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(54) Title: 21 HUMAN SECRETED PROTEINS

(57) Abstract: The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

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21 Human Secreted Proteins

Field of the Invention

This invention relates to newly identified polynucleotides, polypeptides
5 encoded by these polynucleotides, antibodies that bind these polypeptides, uses of
such polynucleotides, polypeptides, and antibodies, and their production.

Background of the Invention

Unlike bacterium, which exist as a single compartment surrounded by a
10 membrane, human cells and other eucaryotes are subdivided by membranes into many
functionally distinct compartments. Each membrane-bounded compartment, or
organelle, contains different proteins essential for the function of the organelle. The
cell uses "sorting signals," which are amino acid motifs located within the protein, to
target proteins to particular cellular organelles.

15 One type of sorting signal, called a signal sequence, a signal peptide, or a
leader sequence, directs a class of proteins to an organelle called the endoplasmic
reticulum (ER). The ER separates the membrane-bounded proteins from all other
types of proteins. Once localized to the ER, both groups of proteins can be further
directed to another organelle called the Golgi apparatus. Here, the Golgi distributes
20 the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes,
and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the
extracellular space as a secreted protein. For example, vesicles containing secreted
proteins can fuse with the cell membrane and release their contents into the
25 extracellular space - a process called exocytosis. Exocytosis can occur constitutively
or after receipt of a triggering signal. In the latter case, the proteins are stored in
secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly,
proteins residing on the cell membrane can also be secreted into the extracellular
space by proteolytic cleavage of a "linker" holding the protein to the membrane.

30 Despite the great progress made in recent years, only a small number of genes
encoding human secreted proteins have been identified. These secreted proteins

include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

Summary of the Invention

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

Detailed Description

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA

preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable
5 of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many
10 mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment,
15 polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain
20 the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the
25 nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the
30 polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig

analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard,
5 Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to
10 sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon
15 sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower
20 percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH₂PO₄; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE,
25 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress
30 background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking

reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA⁺ sequences (such as any 3' terminal polyA⁺ tract of a cDNA shown in the sequence listing), or to a
5 complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any
10 polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA
15 that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for
20 example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide
25 isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications
30 can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in

a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Polynucleotides and Polypeptides of the Invention**FEATURES OF PROTEIN ENCODED BY GENE NO: 1**

5 The translation product of this gene shares sequence homology with claudin-7 from the mouse (*Mus musculus*)(See Genbank Accession gi|4191358; all references available through this accession are hereby incorporated by reference herein) which is thought to be a 4 transmembrane domain receptor involved in formation and maintenance of tight junctions.

10 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:
MVVAVLLGFVAMVLSVVGMMKCTRVGDSNPIAKGRVAIAGGALFILAGLCTL
TAVSWYATLVTXEFFNPSTPVNARYEFGPALFVGXDSAGLAVLSGSFLCCTC
PEPERPNSSPQALSAWTLCCC (SEQ ID NO: 125). Moreover, fragments and
15 variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are
20 also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

 This gene is expressed primarily in human kidney (both fetal and adult) and to a lesser extent in early stage human lung, subtracted library.

 Polynucleotides and polypeptides of the invention are useful as reagents for
25 differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: kidney and lung diseases, especially cancer and other proliferative disorders of the kidneys and lungs. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential
30 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the renal and cardiovascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain

tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 68 as residues: Pro-104 to Pro-120. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution and homology to claudin-7 indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other proliferative disorders, particularly of the kidney and lungs. More specifically, the tissue distribution in kidney indicates the protein product of this clone could be used in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene may be useful for the diagnosis, prevention, and treatment of a variety of respiratory system disorders such as infections, inflammations, ulcers, hereditary disorders, and cancers of the lungs, for example, but not limited to: lung cancer (such as squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma); allergic reactions (such as eosinophilic pneumonia, hypersensitivity pneumonitis, extrinsic allergic alveolitis, allergic interstitial pneumonitis, organic dust pneumoconiosis, allergic bronchopulmonary aspergillosis,

asthma, Wegener's granulomatosis (granulomatous vasculitis), Goodpasture's syndrome)); pneumonia (as may result from bacterial, viral, or fungal pathogens); pleurisy (including for example, pleural effusion and pneumothorax); cystic fibrosis; obstructive airway diseases (such as asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic or acute bronchitis); occupational lung diseases (e.g., silicosis, black lung (coal workers' pneumoconiosis), asbestosis, berylliosis, occupational asthma, byssinosis, and benign pneumoconioses); infiltrative lung diseases (such as pulmonary fibrosis (e.g., fibrosing alveolitis, usual interstitial pneumonia), idiopathic pulmonary fibrosis, desquamative interstitial pneumonia, and lymphoid interstitial pneumonia); acute respiratory distress syndrome (also called, e.g., adult respiratory distress syndrome); edema; pulmonary embolism; bronchitis (e.g., viral, bacterial); bronchiectasis; atelectasis; and lung abscesses (such as may be caused by *Staphylococcus aureus* or *Legionella pneumophila*).

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2458 of SEQ ID NO:11, b is an integer of 15 to 2472, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

25

FEATURES OF PROTEIN ENCODED BY GENE NO: 2

The polypeptide of this gene has been determined to have multiple transmembrane spanning domains. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to multiple transmembrane proteins such as seven transmembrane protein G-coupled receptors.

30

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

MNLSTALLFLNLLFLLDGWITSFN

VDGLCIAVAVLLHFFLLATFTWMGLEAHMYIALVKVFNTYIRRYILKFCIIG

WGLPALVVSVVLASRNNNEVYGKESYGKEKGDEFCWQDPVIFYVTCAGYF

GVMXFLNIAMXIVVMVQICGRNGKRSNRTLREEVVR

5 NLRXVXSLTFLVGMTWX (SEQ ID NO: 91). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the
10 invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in placenta, total fetus, and fetal liver/spleen, and to a lesser extent in a variety of normal and transformed cell and tissue types from
15 fetal and adult sources.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to disorders of the liver and spleen, as well as cancer and other proliferative disorders.
20 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive, hepatic, and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g.,
25 cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively
30 consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 69 as residues: Arg-92 to Glu-110, Gly-148 to Arg-159. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution (with a strong component in rapidly proliferating fetal and placental tissue) and homology to seven transmembrane proteins indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other proliferative disorders. The expression within fetal
5 tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern
10 formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies
15 early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to
20 control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or
25 proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a
30 nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. In addition the expression in fetus would suggest a useful role for the protein

product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue trauma.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
5 related to SEQ ID NO:12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
10 general formula of a-b, where a is any integer between 1 to 4023 of SEQ ID NO:12, b is an integer of 15 to 4037, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.

15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 3**

The protein encoded by this gene is homologous to the mouse IRE1 protein (see Genbank Accession No: AAC64400; all references available through this accession are hereby incorporated herein by reference; for example Wang, X.Z., et al., EMBO J., Oct 1;17(19):5708-17 (1998)). Mouse IRE1, like the yeast IRE1
20 homologue, is believed to be an important cell signal transducing protein. In particular, yeast IRE1, is an essential upstream component of the ER stress-response. The mammalian Ire1 is located in the ER membrane and its over-expression in mammalian cells activates both the endogenous ER chaperone GRP78/BiP and CHOP-encoding genes. Over-expression of murine Ire1 also leads to the
25 development of programmed cell death in transfected cells, indicating that the single upstream component Ire1 plays a role in multiple facets of the ER stress-response in mammalian cells.

Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with IRE1 proteins. Such
30 activities are known in the art, some of which have been described above and elsewhere herein. A preferred polypeptide fragment of the invention comprises, or alternatively consists of, the following amino acid sequence:

MASAVRGSRPWPRGLGLQLQFAALLGLSLXQVHTLRPENLLLSTLDGSLHA
LSKQTGDLKWTLRDDPVIEGPMYVTEMAFLSDPADGSLYLGTQKQQGLMK
LPFTIPELVHASPCRSSDGVFYTGKQDAWFVVDPESETQMTLTTEGPSTPR
LYIGRTQYTVTMHDPRAPALRWNTTYRRYSTPPMDGSTGKYMSQLGVLREG
5 PAAHXGTPGSGTXLLDTRNLGRALGNGPATPLGTKXRAW(SEQIDNO:126)

and

MELALRRSPVPRWLLLLPLLLGLNAGAVIDWPTEEGKEVWDYVTVRKDAY
MFWWLYYATNSCKNFSELPLVMWLQGGPGGSSTGFGNFEEIGPLDSDLKLR
KTTWLQAASLLFVDNPVGTGFSYVNGSGAYAKDLAMVASDMMVLLKTFFS
10 CHKEFQTVPFYIFSESYGGKMAAGIGLELYKAIQRGTIKCNFAGVALGDSWIS
PVDSVLSWGPYLYSMSLLEDKGLAEVSKVAEQVLNAVNGLYREATLWG
KAEMIEQNTDGVNFYNILTKSTPTSTMESSLEFTQSHLVCLCQRHVRHLQRD
ALSQLMNGPIRKKLKIIPEDQSWGGQATNVFVNMEEDFMKPVISIVDELLEAG
INVTVYNGQLDLIVDTMGQEAWVRKLKWPELPKFSQLKWKALYSDPKSLET
15 SAFVKSYSYNLAFYWILKAGHMVPSDQGDMMALKMMRLVTQQE (SEQ ID NO:
127). Polynucleotides encoding these polypeptides are also encompassed by the
invention.

This gene is expressed primarily in colon and cancerous tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for
20 differential identification of the tissue(s) or cell type(s) present in a biological sample
and for diagnosis of diseases and conditions which include but are not limited to
carcinogenesis and tumorigenesis, particularly colon cancer. Similarly, polypeptides
and antibodies directed to these polypeptides are useful in providing immunological
probes for differential identification of the tissue(s) or cell type(s). For a number of
25 disorders of the above tissues or cells, particularly of the immune and digestive
systems, expression of this gene at significantly higher or lower levels may be
routinely detected in certain tissues or cell types (e.g., cancerous and wounded
tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or
another tissue or sample taken from an individual having such a disorder, relative to
30 the standard gene expression level, i.e., the expression level in healthy tissue or bodily
fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 70 as residues: Arg-6 to Trp-11, Ser-54 to Asp-59, Thr-95 to Gly-100, Pro-116 to Asp-121, Thr-126 to Asp-131, Pro-138 to Thr-143, Thr-149 to Pro-155, Thr-180 to Lys-196.

5 Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution and homology to protein kinase indicates that polynucleotides and polypeptides corresponding to this gene are useful for colon cancer. The expression within cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility
10 in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure
15 to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate
20 with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating,
25 detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in
30 proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in.

addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly
5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:13 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
10 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1579 of SEQ ID NO:13, b is an integer of 15 to 1593, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

15

FEATURES OF PROTEIN ENCODED BY GENE NO: 4

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:
VRGGGHSRPLSALEERKTDSYRYPRTGSKNPKEALKLAEFQTDSQGKIVSTQE
20 KELVQPFSSLFPKVEYIARAGWTRDGKYAWA (SEQ ID NO: 128) and
MFLDRPQQWLQLVLLPPALFIPSTENEEQRLASARAVPRNVQPYVVYEEVTN
VWINVHDIFYPFPQSEGEDELCLFLRANECKTGFCCHLYKVTAVLKSQGYDWSE
PFSPGEDEFKCPIKEELALTSGEWEVLARHGSKIWVNEETKLVYFQGTKDTPL
EHHLYVVS YEAAAGEIVRLTTPGF SHXCSMSQNFXXFVSHITAQVAAASAGNQ
25 AGGTEWPAGPSEALCPAQRWPAPRSRCLHRPDAFYFPLNALGFYVRCFLVAE
TERWWSRASPSRPRLGGGGHTLMGTGEARRDSEERA AFRLGLPVTSQSPGP
ASHRPQHPSMQLPVPPGQPPXLDVCVLFGGXXFIXI (SEQ ID NO: 129).
Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,
30 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind

polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Primary Dendritic Cells, lib 1, Soares senescent fibroblasts NbHSF, Soares fetal liver spleen 1NFLS S1, and Soares NFL T
 5 GBC S1, fetal kidney (reexcision) and Activated T-cell(12h)/Thiouridine-re-excision; and to a lesser extent in a variety of normal and transformed tissues and cell types. The list of cell types in which expression of this gene has been observed is (in order of precedence): Primary Dendritic Cells, lib
 1;Soares_senescent_fibroblasts_NbHSF;Soares_fetal_liver_spleen_1NFLS_S1;Soares
 10 s_NFL_T_GBC_S1;Human Fetal Kidney; Reexcision;Activated T-cell(12h)/Thiouridine-re-excision;Adipocytes;re-excision;Human umbilical vein endothelial cells, IL-4 induced;Human Chondrosarcoma;Epithelial-TNF α and INF induced;NCI_CGAP_Pan1;Smooth muscle, serum induced,re-exc;Macrophage-oxLDL; re-excision;CHME Cell Line;treated 5 hrs;Adipocytes;CD34 positive cells
 15 (Cord Blood);Human Thymus Stromal Cells;T Cell helper I;NCI_CGAP_Lu5;PC3 Prostate cell line;Soares_parathyroid_tumor_NbHPA;Soares melanocyte 2NbHM;Soares_fetal_heart_NbHH19W;Kidney cancer;Human Thymus Tumor, subtracted;Tongue Tumour;NCI_CGAP_Co4;Human Umbilical Vein Endothelial cells, frac B, re-excision;Lung, Normal: (4005313 B1);Human OB HOS control
 20 fraction I;Human OB MG63 treated (10 nM E2) fraction I;NCI_CGAP_Br1.1;Human Pituitary, subtracted;Aorta endothelial cells + TNF- α ;Smooth muscle, control; re-excision;NCI_CGAP_AA1;Human adult small intestine,re-excision;Human Quadriceps;STROMAL -OSTEOCLASTOMA;Human Pre-Differentiated Adipocytes;NCI_CGAP_Co10;NCI_CGAP_Co14;Human Osteosarcoma;HL-60,
 25 PMA 4H, re-excision;NCI_CGAP_Pr12;H. Lymph node breast Cancer;CD34 depleted Buffy Coat (Cord Blood);Human Umbilical Vein; Reexcision;NCI_CGAP_Pr22;Monocyte activated; re-excision;KMH2;NCI_CGAP_Gas4;Human Prostate Cancer, Stage B2; re-excision;B-Cells;NCI_CGAP_Br2;Spinal cord;NCI_CGAP_Co3;Fetal Heart;Ovarian Tumor 10-
 30 3-95;Rejected Kidney, lib 4;Brain frontal cortex;B-cells (unstimulated);Endothelial-induced;Myeloid Progenitor Cell Line;Primary Dendritic cells,frac 2;NCI_CGAP_Brn25;Pancreas Islet Cell Tumor;Human Microvascular Endothelial

Cells, fract. A; Monocyte activated; Osteoblasts; Colon Tumor II;
Soares_pregnant_uterus_NbHPU;
Soares_NhHMPu_S1; Soares_testis_NHT; NCI_CGAP_GCB1; NCI_CGAP_Sub3
cells.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for
10 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system (especially spleen, dendritic cells and other B-cells), liver and testes, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma,
15 urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively
20 consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 71 as residues: Asp-4 to Trp-9, Thr-24 to Arg-30, Pro-65 to Glu-70, Ser-97 to Glu-112, Gln-150 to Pro-156, Cys-184 to Phe-190, Leu-211 to Arg-224, Gln-266 to His-271, Gly-313 to Gly-320, Asp-397 to Glu-402, Pro-408 to Gly-414, Lys-426 to Asn-432, Pro-470 to Arg-477, Pro-479 to His-484. Polynucleotides encoding said polypeptides
25 are also encompassed by the invention.

The tissue distribution (found in rapidly proliferating cells such as fibroblasts, fetal tissues, and primary dendritic cells) indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other
30 proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the

development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis.

- 5 Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type
- 10 specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases.
- 15 The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore; the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.
- 20 Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of

25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2938 of SEQ ID NO:14, b

30 is an integer of 15 to 2952, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 5

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

5 MKVLATSFVLGSLGLAFYLPLVVTTTPKTLAIPKQLQEAVGKVIINATTCTVTC
GLGYKEETVCEVGPDGVRRKCQTRRLECLTNWICGMLHFTILIGKEFELSCLS
SDILEFGQEAFRFTXXLARGVISTDDEVFKPFQANSHFVKFKYAEYDSGTYS
CDVQLVKNLRLVKSSILG (SEQ ID NO: 130). Moreover, fragments and variants
of these polypeptides (such as, for example, fragments as described herein,
10 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,
under stringent conditions, to the polynucleotide encoding these polypeptides) are
encompassed by the invention. Antibodies that bind polypeptides of the invention are
also encompassed by the invention. Polynucleotides encoding these polypeptides are
15 also encompassed by the invention.

This gene is expressed primarily in IL-1 and LPS induced Neutrophils and to a lesser extent in fetal heart.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample
20 and for diagnosis of diseases and conditions which include but are not limited to
disorders of the heart, disorders of development, and immunological disorders (for
example, arthritis), particularly disorders involving neutrophils. Similarly,
polypeptides and antibodies directed to these polypeptides are useful in providing
immunological probes for differential identification of the tissue(s) or cell type(s). For
25 a number of disorders of the above tissues or cells, particularly of the immune system
and cardiovascular system, expression of this gene at significantly higher or lower
levels may be routinely detected in certain tissues or cell types (e.g., cancerous and
wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal
fluid) or another tissue or sample taken from an individual having such a disorder,
30 relative to the standard gene expression level, i.e., the expression level in healthy
tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 72 as residues: Pro-67 to Arg-78, Glu-152 to Arg-159, Ser-193 to Leu-200, Asp-210 to Lys-223. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates the polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a variety of immune system disorders. For example, the expression pattern indicates this gene and/or gene product may play a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes suggests a usefulness for treatment of cancer (e.g. by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it would also be useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. The expression within fetal tissue and other cellular sources marked by proliferating

cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue trauma.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
 5 general formula of a-b, where a is any integer between 1 to 1339 of SEQ ID NO:15, b is an integer of 15 to 1353, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

10 FEATURES OF PROTEIN ENCODED BY GENE NO: 6

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:
 NIFLEWILRRILSLWRGTFLMHGRAGVNRISYWPADPEISLLTEASSSEDAKLD
 AKAVERLKSNSRAHVCVLLQPLVCYMQFVEETSYKCDFIQKITKTLPDANT
 15 DFYYECKQERIKEYEMLKKKKKKKT (SEQ ID NO: 131). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these
 20 polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Human adult (K.Okubo);Jurkat T-cell G1 phase;Stratagene neuroepithelium (#937231);Healing groin wound, 6.5 hours post
 25 incision;12 Week Early Stage Human II; Reexcision;Colon Tumor II;Soares_NFL_T_GBC_S1

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to
 30 cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of

the above tissues or cells, particularly of epithelial tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. The protein product of this gene (expressed via a transient transfection) has been shown to caused a positive result in an EGR assay in sensory neurons.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 73 as residues: Ala-28 to His-41, Pro-43 to Lys-60. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The expression within cellular sources marked by rapidly proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of

degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate
5 cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly
10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
15 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1778 of SEQ ID NO:16, b is an integer of 15 to 1792, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 7

This gene is expressed primarily in human fetal kidney; reexcision.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample
25 and for diagnosis of diseases and conditions which include but are not limited to: kidney disorders and diseases, including cancer and other proliferative disorders of the kidney. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,
30 particularly of the renal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and

spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively
5 consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 74 as residues: Leu-63 to Gly-73. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other
10 proliferative disorders, particularly of the kidney. More specifically, the tissue distribution in kidney indicates the protein product of this clone could be used in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney
15 stones, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as,
20 antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of
25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 655 of SEQ ID NO:17, b
30 is an integer of 15 to 669, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 8

This gene is expressed primarily in colon adenocarcinoma, normalized infant brain, breast, total fetus, pancreas, pregnant uterus, and infant brain and to a lesser extent in a variety of normal and transformed tissues and cells. The complete list of tissues and cells in which this gene is expressed (in order of precedence) is

NCI_CGAP_Co8;normalized infant brain cDNA;Soares infant brain
 1NIB;Soares_total_fetus_Nb2HF8_9w;Soares breast
 3NbHBst;NCI_CGAP_Pan1;Soares_pregnant_uterus_NbHPU;Stratagene endothelial
 cell 937223;NCI_CGAP_GC6;NCI_CGAP_Kid8;Human Skin
 Tumor;NCI_CGAP_Alv1;Human umbilical vein endothelial cells, IL-4 induced;Fetal
 Liver, subtraction II;Human endometrial stromal cells-treated with progesterone;12
 Week Early Stage Human II; Reexcision;Endothelial-
 induced;NCI_CGAP_Kid5;Soares_multiple_sclerosis_2NbHMSP;T Cell helper
 I;Soares melanocyte 2NbHM;Soares_NFL_T_GBC_S1;Soares placenta
 Nb2HP;Soares_fetal_heart_NbHH19W;NCI_CGAP_GCB1;Lung
 Mesothelium;Ea.hy.926 cell line;Human Prostate, subtracted;Normalized infant brain,
 Bento Soares;Human Colon;H. Atrophic Endometrium;Human Umbilical Vein
 Endothelial Cells, fract. A;Human fetal brain (TFujiwara);Human
 Placenta;NCI_CGAP_Lu1;Fetal Heart, re-excision;Stromal cells
 3.88;NCI_CGAP_Ut4;Messangial cell, frac 2;Human endometrial stromal cells-
 treated with estradiol;Glioblastoma;Human Pre-Differentiated Adipocytes;Human
 endometrial stromal cells;NCI_CGAP_Lym12;Colon, tumour;Spleen metastatic
 melanoma;Prostate BPH;Human Infant Brain;Brain Frontal Cortex, re-
 excision;Human Prostate;Gessler Wilms tumor;NCI_CGAP_Ut1;Human T-cell
 lymphoma;re-excision;12 Week Old Early Stage Human, II;Human Umbilical Vein
 Endothelial Cells, uninduced;Stromal cell TF274;Human
 Hypothalmus,Schizophrenia;Ovary, Cancer: (4004562 B6) Papillary Serous Cystic
 Neoplasm, Low Malignant
 Pot;NCI_CGAP_Br2;Soares_NSF_F8_9W_OT_PA_P_S1;Colon Normal;Spinal
 cord;Human Chondrosarcoma;Epithelial-TNFa and INF induced;Stratagene hNT
 neuron (#937233);Ovary, Cancer: (4004576 A8);Human Placenta (re-

excision);NTERA2 + retinoic acid, 14 days;CHME Cell Line,untreated;Ovarian Tumor 10-3-95;Smooth muscle, serum treated;NCI_CGAP_Kid11;NCI_CGAP_GC4;Early Stage Human Brain;Human Testes Tumor;Colon Normal II;Liver Normal Met5No;Ovary, Cancer (9809C332):

5 Poorly differentiated adenocarcinoma;Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma;Normal colon;NTERA2, control;Myeloid Progenitor Cell Line;Human Osteoclastoma;Human Fetal Heart;Endothelial cells-control;Stratagene lung (#937210);Human Adult Pulmonary; re-excision; NCI_CGAP_Brn23;Soares_placenta_8to9weeks_2NbHP8to9W ;Human

10 Testes;Osteoblasts;Hodgkin's Lymphoma II;Human 8 Week Whole Embryo;Nine Week Old Early Stage Human;Colon Tumor II;T cell helper II;Colon Normal III;NCI_CGAP_HN5;NCI_CGAP_CML1;NCI_CGAP_Co17;NCI_CGAP_Co21

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

15 and for diagnosis of diseases and conditions which include but are not limited to cancer and other cell proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the colon, brain and breast, expression of this

20 gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an

25 individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 75 as residues: Met-1 to Tyr-7. Polynucleotides encoding said polypeptides are also encompassed by the invention.

30 The expression within cellular sources marked by rapidly proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases

and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue trauma.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3800 of SEQ ID NO:18, b is an integer of 15 to 3814, where both a and b correspond to the positions of
 5 nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 9

In specific embodiments, polypeptides of the invention comprise, or
 10 alternatively consists of, the following amino acid sequence:
 MTSVSQASLDVSMIIISLGAICAVLLVIMVLFATRCNREKKDTRSYNCRVAES
 TYQHHPKRPSRQIHKGDITLVPTINGTLPIRSHHRSSPSSSPTLERGQMGSRQSH
 NSHQSLNSLVTISSNHVPENFSLELTHATPAVERLSASFNASPGAISAKTKFSR
 KQIFQELQICPSRHGQI (SEQ ID NO: 132). Moreover, fragments and variants of
 15 these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are
 20 also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in rapidly proliferating tissues including Morton Fetal Cochlea, Human 8 Week Whole Embryo, 12 Week Old Early Stage Human, and brain glioblastoma (NCI_CGAP Brn23) and to a lesser extent in a variety
 25 of normal and transformed fetal and adult tissues. The complete list of libraries/tissues in which expression of this gene has been observed is (in order of precedence): Morton Fetal Cochlea; Human 8 Week Whole Embryo; 12 Week Old Early Stage Human; NCI_CGAP_Brn23; Human endometrial stromal cells; Synovial hypoxia; Synovial Fibroblasts (control); Bone Marrow Stromal Cell,
 30 untreated; NCI_CGAP_Co8; Osteoblasts; Soares_fetal_liver_spleen_1NFLS_S1; Soares placenta Nb2HP; Soares_fetal_heart_NbHH19W; Soares_NhHMPu_S1; Stratagene cat#937212 (1992); Human White Adipose; Human Fetal Spleen; Human fetal brain

(TFujiwara);NCI_CGAP_Co12;HSA 172 Cells;NCI_CGAP_AA1;Human adult
 (K.Okubo);NCI_CGAP_Co9;Salivary Gland, Lib 2;Stratagene lung carcinoma
 937218;NCI_CGAP_Kid6;human ovarian
 cancer;NCI_CGAP_Pr28;NCI_CGAP_Pan1;Human T-Cell Lymphoma;Human
 5 endometrial stromal cells-treated with progesterone;Human Testes Tumor;Human
 Pancreas Tumor; Reexcision;Human Fetal Kidney; Reexcision;Human Testes,
 Reexcision;12 Week Early Stage Human II; Reexcision;Pancreas Islet Cell
 Tumor;Prostate
 Adenocarcinoma;Soares_total_fetus_Nb2HF8_9w;Soares_pregnant_uterus_NbHPU;
 10 Soares_NFL_T_GBC_S1;NCI_CGAP_Co17.

Polynucleotides and polypeptides of the invention are useful as reagents for
 differential identification of the tissue(s) or cell type(s) present in a biological sample
 and for diagnosis of diseases and conditions which include but are not limited to:
 cancer and other cell proliferative disorders. Similarly, polypeptides and antibodies
 15 directed to these polypeptides are useful in providing immunological probes for
 differential identification of the tissue(s) or cell type(s). For a number of disorders of
 the above tissues or cells, particularly during fetal development, and in the brain and
 cochlea (and other neural tissue), expression of this gene at significantly higher or
 lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous
 20 and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and
 spinal fluid) or another tissue or sample taken from an individual having such a
 disorder, relative to the standard gene expression level, i.e., the expression level in
 healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively
 25 consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 76 as
 residues: Arg-36 to Asn-48, Pro-60 to Gly-70, Arg-85 to Ser-94, Pro-96 to Ser-114,
 His-151 to Ser-169, Lys-183 to Pro-204, Pro-249 to Asn-258, Glu-265 to Ser-294,
 Phe-298 to Thr-313, Ser-331 to Gly-352, His-373 to Cys-381, Ser-389 to Lys-396,
 Glu-403 to Phe-413. Polynucleotides encoding said polypeptides are also
 30 encompassed by the invention.

The expression within fetal tissue and other cellular sources marked by rapidly
 proliferating cells indicates this protein may play a role in the regulation of cellular

division, and are useful for the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can
5 result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant
10 expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore,
15 the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in
20 modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed
25 against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of
30 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2823 of SEQ ID NO:19, b is an integer of 15 to 2837, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 10

This gene is expressed primarily in prostate cancer.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to disorders of the prostate and, in particular, prostate cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 77 as residues: Arg-43 to Cys-48. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, prevention, and or treatment of disorders of the prostate (for example, but not limited to acute prostatitis, chronic prostatitis, benign prostatic hypertrophy, and prostate cancer).

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the
5 general formula of a-b, where a is any integer between 1 to 1491 of SEQ ID NO:20, b is an integer of 15 to 1505, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

10 FEATURES OF PROTEIN ENCODED BY GENE NO: 11

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

MAVYVGMLRLGRLCAGSSGVLGARAALSRSWQEARLQGVRFLSSREVD
VSTPIGGLSYVQGCTKKHLNSKTVGQCLETTAQRVPEREALVVLHEDVRLTF
15 AQLKEEVDKAASGLLSIGLCKGDRLGMWGPNSYAWVLXQLATGQAGIILVS
VXPAYQAMEWSXSSKKWASXALVVPKQFKTKHNTTFLKQIXPXWRMP

(SEQ ID NO: 133). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides
20 encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

25 This gene is expressed primarily in eosinophils and to a lesser extent in fetal heart and kidney.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to
30 immunological disorders (particularly those involving eosinophils, for example, inflammation), disorders involving the heart, kidneys, and organ development. Similarly, polypeptides and antibodies directed to these polypeptides are useful in

providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, renal and cardiovascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g.,
5 cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively
10 consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 78 as residues: Arg-29 to Arg-35, Cys-64 to Thr-73, Thr-81 to Glu-87, Lys-123 to Met-129, Lys-179 to Val-189, Ser-237 to Gln-250, Ser-264 to Lys-271, His-294 to Gln-300, Ser-346 to Thr-351. Polynucleotides encoding said polypeptides are also encompassed by the invention.

15 The tissue distribution indicates the polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a variety of immune system disorders. For example, the expression pattern indicates this gene and/or gene product may play a role in regulating the proliferation, survival, differentiation, and/or activation of hematopoietic cell lineages, including blood stem
20 cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes suggests a usefulness for treatment of cancer (e.g. by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it would also be useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency
25 diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue
30 injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other

blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

5 The tissue distribution in kidney indicates the protein product of this clone could be used in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilm's Tumor Disease, and congenital
10 kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome.

 The expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of
15 developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in
20 acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers.

25 Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types
30 of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in

modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues.

In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue trauma. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1494 of SEQ ID NO:21, b is an integer of 15 to 1508, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 12

This gene is expressed primarily in immune/hematopoietic cells such as macrophages (H Macrophage (GM-CSF treated), re-excision; Macrophage-oxLDL, re-excision; Macrophage-oxLDL; Macrophage; GM-CSF treated) and to a lesser extent in kidney, colon, thymus, eosinophils, activated monocytes, bone marrow, melanocytes and primary dendritic cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: immunological disorders (for example, AIDS, lupus, leukemia, and arthritis).

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 79 as residues: Ser-2 to Trp-7, Gln-44 to Lys-53, Ser-80 to Gly-88. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for modulating immune response, particularly of macrophages, monocytes, melanocytes, and primary dendritic cells. This could be useful in the treatment of a variety of immune system disorders. For example, the expression pattern indicates this gene and/or gene product may play a role in regulating the proliferation, survival, differentiation, and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes suggests a usefulness for treatment of cancer (e.g. by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it would also be useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that

influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 771 of SEQ ID NO:22, b is an integer of 15 to 785, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 13

This gene is expressed primarily in prostate cancer.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis, prevention, and/or treatment of diseases and conditions which include but are not limited to disorders of the prostate and, in particular, prostate cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g.,

cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, prevention, and or treatment of disorders of the prostate (for example, but not limited to acute prostatitis, chronic prostatitis, benign prostatic hypertrophy, and prostate cancer).

10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

15 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 500 of SEQ ID NO:23, b is an integer of 15 to 514, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

20

FEATURES OF PROTEIN ENCODED BY GENE NO: 14

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

MVVMASLQVEPAVGKEQLRERQGPELLGWVAGLAFVCLFACVGVGVPCH
25 SFDSEAASFLLLYSWCTPRLXSWLRDTPSPLASGTXP (SEQ ID NO: 110).

Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide

30 encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in Activated T-cells and to a lesser extent in myeloid Progenitor Cell Line and in testis tumor.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to immunological disorders (such as immune deficiencies and leukemia). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 81 as residues: Glu-16 to Pro-24. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The expression of this gene in T-cells suggests a potential role in the treatment/detection of immune disorders such as arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Furthermore, expression of this gene in T-cells as well as other immune cells also indicates the polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a wide variety of immune system disorders. For example, the expression pattern indicates this gene and/or gene product may play a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes suggests a usefulness for treatment of cancer (e.g. by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it would also be useful as an agent for immunological disorders

including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

The tissue distribution in testis tumors indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of tumor progression. Moreover, expression of this gene product in the testis and testicular cancer tissue may implicate this gene product in normal testicular function. In addition, this gene product would be useful in the treatment of male infertility, and/or could be used as a male contraceptive. Therefore, polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product would be useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that is expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2272 of SEQ ID NO:24, b is an integer of 15 to 2286, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 15

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

MGPRGCALAHSLPLLCQHVTSPRYCRQCTREPRHCCPAPASAGVQYMCA
YGCHHPTFAGVYTPSHTTVATSICTQTPPHQCCWSEHTHVVSTTPLLPAVMH
MSMDPAATTQMKCFCHRPIRAFLPVEWEHLSPFNTAXA (SEQ ID NO: 134).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in prostate cancer tissue.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to disorders of the prostate and, in particular, prostate cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely

detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 82 as residues: Trp-21 to Cys-38, Gln-77 to Trp-85. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, prevention, and or treatment of disorders of the prostate (for example, but not limited to acute prostatitis, chronic prostatitis, benign prostatic hypertrophy, and prostate cancer).

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 465 of SEQ ID NO:25, b is an integer of 15 to 479, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 16

This gene is expressed primarily in brain and to a lesser extent in prostate cancer.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to neurological disorders (for example, neuronal degeneration). Similarly, polypeptides

and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain
5 tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 83 as residues: Ser-18 to Arg-25. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in brain indicates polynucleotides and polypeptides
15 corresponding to this gene would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. For example, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, epilepsy, meningitis, encephalitis,
20 demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and
25 perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Elevated levels of expression of this protein in prostate cancer indicate polynucleotides and polypeptides
30 corresponding to this gene would be useful for the diagnosis, prevention, and or treatment of disorders of the prostate (for example, but not limited to acute prostatitis, chronic prostatitis, benign prostatic hypertrophy, and prostate cancer).

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 630 of SEQ ID NO:26, b is an integer of 15 to 644, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 17

The protein product of this gene shares a discrete region of high sequence similarity with ubiquitin-activating enzyme (see Genbank Accession gb|AAA616246; all references available through this accession are hereby incorporated by reference herein) which is thought to be involved in regulating protein turnover in cells. The homology suggests that the gene product of the present invention represents a novel gene encoding a functional domain shared in common with ubiquitin-activating enzyme.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: MVPIFLLKCLLLHVPLCMSSNLSFHSSHHLHIFLPSFSSHLPRPLXIPPLSP (SEQ ID NO: 136) and

MYPIDFEKDDDSNFHMDFIVAASNLRANEDIPSADRHKSKLIAGKIIPAIATT
TAAVVGLVCLELYKVVQGHRQLDSYKNGFLNLALPFFGFSEPLAAPRHQYY
NQEWTWDRFEVQGLQPNGEEMTLKQFLDYFKTEHKLEITMLSQGVSMMLYS
FFMPAAKLKERLDQPMTEIVSRVSKRKLGRHVRAVLLELCCNDESGEDVEVP
YVRYTIR (SEQ ID NO: 135). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent

conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for
10 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an
15 individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other
20 proliferative disorders. The expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell
25 differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the
30 pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers.

Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, 5 detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in 10 proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets 15 for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically 20 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1789 of SEQ ID NO:27, b is an integer of 15 to 1803, where both a and b correspond to the positions of 25 nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 18

In specific embodiments, polypeptides of the invention comprise, or 30 alternatively consists of, the following amino acid sequence:
MELSCPGSRCVPVQEQRARWERKRACTARELLETERRYQEQLGLVATYFLGIL
KAKGTLRPPERQALFGSWELIYGASQELLPYLEGGCWGQGLEGFCRHLELYN

QFAANSERSQTXLQEQLKKNKGFRKFVRLQEGRPEFGGLQLQDLLPLPLQRL
QQYENLVVALAENTGPNSPDHQQQLTRRFLLLGNAGWRLPLLYSFLILTSNNV
WYDPIFH (SEQ ID NO: 137). Moreover, fragments and variants of these

polypeptides (such as, for example, fragments as described herein, polypeptides at
5 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides
and polypeptides encoded by the polynucleotide which hybridizes, under stringent
conditions, to the polynucleotide encoding these polypeptides) are encompassed by
the invention. Antibodies that bind polypeptides of the invention are also
encompassed by the invention. Polynucleotides encoding these polypeptides are also
10 encompassed by the invention.

This gene is expressed primarily in Fetal heart, re-excision; TF-1 Cell Line
GM-CSF Treated; Human Ovary; Keratinocyte; NCI_CGAP_GCB1 (Germinal
Center B-cell) and Primary Dendritic Cells, lib 1.

Polynucleotides and polypeptides of the invention are useful as reagents for
15 differential identification of the tissue(s) or cell type(s) present in a biological sample
and for diagnosis of diseases and conditions which include but are not limited to
cancer and other proliferative disorders. Similarly, polypeptides and antibodies
directed to these polypeptides are useful in providing immunological probes for
differential identification of the tissue(s) or cell type(s). For a number of disorders of
20 the above tissues or cells, particularly of the immune system, expression of this gene
at significantly higher or lower levels may be routinely detected in certain tissues or
cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma,
urine, synovial fluid and spinal fluid) or another tissue or sample taken from an
individual having such a disorder, relative to the standard gene expression level, i.e.,
25 the expression level in healthy tissue or bodily fluid from an individual not having the
disorder.

The expression within fetal tissue and other cellular sources marked by
proliferating cells indicates this protein may play a role in the regulation of cellular
division, and may show utility in the diagnosis, treatment, and/or prevention of
30 developmental diseases and disorders, including cancer, and other proliferative
conditions. For example, developmental tissues rely on decisions involving cell
differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can

result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1914 of SEQ ID NO:28, b is an integer of 15 to 1928, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.

FEATURES OF PROTEIN ENCODED BY GENE NO: 19

5 In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

MALLADCALPPELRGDLWELPFPCPDGFNSCPDICFRVAGCSFLCHKAFFCGR
SDYFRALLDDHFRESEEPATSGGPPAVTLHGISP DVFTHVLYYMYSDHTELSP
EAAYDVLSVADMYLLPGLKRLCGRSLAQMLDEDTVVG VWRVAKLFRRLARL
10 EDQCTEYMAKVIEKLVEREDFVEAVKEEAAAVAARQETDSIPLVDDIRFHVA
STVQTYSAIEEAQQRLRALEDLLVSIGLDC (SEQ ID NO: 138). Moreover,
fragments and variants of this polypeptide (such as, for example, fragments as
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or
99% identical to these polypeptides and polypeptides encoded by the polynucleotide
15 which hybridize, under stringent conditions, to the polynucleotide encoding this
polypeptide are encompassed by the invention. Antibodies that bind polypeptides of
the invention are also encompassed by the invention. Polynucleotides encoding this
polypeptide are also encompassed by the invention.

20 This gene is expressed primarily in breast lymph node and to a lesser extent in
colon tumor and B-cell lymphoma.

Polynucleotides and polypeptides of the invention are useful as reagents for
differential identification of the tissue(s) or cell type(s) present in a biological sample
and for diagnosis of diseases and conditions which include but are not limited to
immunological disorders, carcinogenesis, and tumorigenesis. Similarly, polypeptides
25 and antibodies directed to these polypeptides are useful in providing immunological
probes for differential identification of the tissue(s) or cell type(s). For a number of
disorders of the above tissues or cells, particularly of the immune system, digestive
system, and mammary glands, expression of this gene at significantly higher or lower
levels may be routinely detected in certain tissues or cell types (e.g., cancerous and
30 wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal
fluid) or another tissue or sample taken from an individual having such a disorder,

relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 86 as
5 residues: Glu-34 to Gly-41, Ile-43 to Gly-62, Pro-85 to Ser-92. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and/or treatment of tumor progression. The expression within cellular sources marked by proliferating cells
10 indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate
15 suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant
20 expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore,
25 the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in
30 modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The tissue distribution also indicates the polynucleotides and polypeptides corresponding to this gene would be useful for the

diagnosis and treatment of a variety of immune system disorders. For example, the expression pattern indicates this gene and/or gene product may play a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes suggests a usefulness for treatment of cancer (e.g. by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it would also be useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 2031 of SEQ ID NO:29, b is an integer of 15 to 2045, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

5

FEATURES OF PROTEIN ENCODED BY GENE NO: 20

A preferred polypeptide fragment of the invention comprises, or alternatively consists of, the following amino acid sequence:

MSSNTMLQKTLLILISFSVVTWMIFIISQNFTKLWSALNLSISVHYWNNSAKSL
10 FPKTSLIPLKPLTETELRIKEIEKLDQQIPRPFTHVNTTTSATHSTATILNPRDT
YCRGDQLDILLEVRDHLGQRKQYGGDFLRARMSFPALTAGASGKVMDFTM
APTWQLHSGLGGPGLPGSXXYSPQVEGAXG (SEQ ID NO: 139).

Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in colon and ulcerative colitis.

15 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to disorders of the gastrointestinal tract, for example ulcerative colitis, colon cancer, and diverticulitis. Similarly, polypeptides and antibodies directed to these polypeptides
20 are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and digestive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma,
25 urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively
30 consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 87 as residues: Tyr-46 to Ala-51, Leu-80 to Phe-89, Pro-108 to Asp-116, His-127 to Gly-136, Ala-195 to Asp-201, Asp-235 to Glu-240, Asn-296 to Ile-303, Trp-398 to Tyr-

403, Leu-426 to Asn-431. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for preventing and/or treating colon cancer and/or ulcerative colitis. The secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, and as nutritional supplements. It may also have a very wide range of biological activities. Representative uses are described in the "Chemotaxis" and "Binding Activity" sections below, in Examples 11, 12, 13, 14, 15, 16, 18, 19, and 20, and elsewhere herein. Briefly, the protein may possess the following activities: cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g., for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds, stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2075 of SEQ ID NO:30, b

is an integer of 15 to 2089, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

5 FEATURES OF PROTEIN ENCODED BY GENE NO: 21

The translation product of this gene shares sequence homology with the human CoREST protein, a functional corepressor required for regulation of neural-specific gene expression. See, Genbank Protein Accession AAF01498; all references available through this accession are hereby incorporated herein by reference, for
10 example, Andres, M.E., et al., Proc. Natl. Acad. Sci. U.S.A. 96: 9873-9878 (1999). Based on sequence similarity with human CoREST, the translation product of this clone is expected to share at least some biological activities with transcriptional regulatory proteins. Such activities are known in the art, some of which are described elsewhere herein.

15 This gene is expressed primarily in pancreas and to a lesser extent in placenta, embryonic tissues, immune cells (such as neutrophils, monocytes, and T-cells), and tumor tissues (such as osteosarcoma and ovarian tumor).

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample
20 and for diagnosis of diseases and conditions which include but are not limited to pancreatic disorders, developmental disorders, and immunological disorders (particularly neutrophils, monocytes, and T-cells). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of
25 disorders of the above tissues or cells, particularly of the pancreas and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to
30 the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 88 as residues: Leu-24 to Ser-30. Polynucleotides encoding said polypeptides are also encompassed by the invention.

5 The expression within fetal tissues other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and are useful for the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. For example, developmental tissues rely on decisions involving cell
10 differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the
15 pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It
20 may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue
25 differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The tissue distribution also indicates the polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a variety of immune system disorders. For example, the
30 expression pattern indicates this gene and/or gene product may play a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation

of cytokine production, antigen presentation, or other processes suggests a usefulness for treatment of cancer (e.g. by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it would also be useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 793 of SEQ ID NO:31, b is an integer of 15 to 807, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

Table 1

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HFKLX38	PTA-2074 06/09/00	Uni-ZAP XR	11	2472	1	2472	73	73	68	1	18	19	128
1	HFKLX38	PTA-2074 06/09/00	Uni-ZAP XR	32	433	1	433	60	60	89	1	18	19	124
1	HFKLX38	PTA-2074 06/09/00	Uni-ZAP XR	33	1009	1	828		1009	90	1	1	2	97
2	HUVFH14	PTA-2074 06/09/00	Uni-ZAP XR	12	4037	1	3996	141	141	69	1	51	52	320
2	HUVFH14	PTA-2074 06/09/00	Uni-ZAP XR	34	683	1	683	141	141	91	1	22	23	181
2	HUVFH14	PTA-2074 06/09/00	Uni-ZAP XR	35	3085	42	873		2520	92	1	1	2	118
3	HWMMAH36	PTA-2074 06/09/00	pSport1	13	1593	1	1593	36	36	70	1	34	35	473
3	HWMMAH36	PTA-2074 06/09/00	pSport1	36	773	1	773	36	36	93	1	34	35	246
4	HUVHI35	PTA-2074 06/09/00	Uni-ZAP XR	14	2952	1	2952	107	107	71	1	26	27	497

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
4	HUVHI35	PTA-2074 06/09/00	Uni-ZAP XR	37	3047	1	2848	197	197	94	1	26	27	497
4	HUVHI35	PTA-2074 06/09/00	Uni-ZAP XR	38	1837	657	1837	756	756	95	1	26	27	349
4	HUVHI35	PTA-2074 06/09/00	Uni-ZAP XR	39	604	199	421		485	96	1	1	2	144
5	HHFML08	PTA-2074 06/09/00	Uni-ZAP XR	15	1353	48	1349	133	133	72	1	30	31	255
5	HHFML08	PTA-2074 06/09/00	Uni-ZAP XR	40	1313	1	1304	93	93	97	1	30	31	255
5	HHFML08	PTA-2074 06/09/00	Uni-ZAP XR	41	1352	48	1348	133	133	98	1	30	31	177
6	HE2KK74	PTA-2074 06/09/00	Uni-ZAP XR	16	1792	1	1792	154	154	73	1	28	29	87
6	HE2KK74	PTA-2074 06/09/00	Uni-ZAP XR	42	1683	1	1683	46	46	99	1	28	29	87
6	HE2KK74	PTA-2074 06/09/00	Uni-ZAP XR	43	982	1	730		394	100	1	1	2	131
7	HFKME15	PTA-2074 06/09/00	Uni-ZAP XR	17	669	1	669	54	54	74	1	19	20	100
7	HFKME15	PTA-2074 06/09/00	Uni-ZAP XR	44	530	1	530	46	46	101	1	19	20	100

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
8	HE2LW65	PTA-2074 06/09/00	Uni-ZAP XR	18	3814	1	3814	16	16	75	1	33	34	94
8	HE2LW65	PTA-2074 06/09/00	Uni-ZAP XR	45	541	1	541	9	9	102	1	33	34	94
8	HE2LW65	PTA-2074 06/09/00	Uni-ZAP XR	46	2183	1	823		1399	103	1	1	2	133
9	HTPHS66	PTA-2074 06/09/00	Uni-ZAP XR	19	2837	1	2837	100	100	76	1	39	40	447
9	HTPHS66	PTA-2074 06/09/00	Uni-ZAP XR	47	2837	1	2837	100	100	104	1	39	40	240
9	HTPHS66	PTA-2074 06/09/00	Uni-ZAP XR	48	2718	1	2718	5	5	105	1	39	40	180
10	HPJEG57	PTA-2074 06/09/00	Uni-ZAP XR	20	1505	1	1505	18	18	77	1	33	34	93
10	HPJEG57	PTA-2074 06/09/00	Uni-ZAP XR	49	571	1	571	18	18	106	1	33	34	93
10	HPJEG57	PTA-2074 06/09/00	Uni-ZAP XR	50	821	1	821		436	107	1	1	2	59
11	HHFKM76	PTA-2074 06/09/00	Uni-ZAP XR	21	1508	1	1508	124	124	78	1	22	23	461
11	HHFKM76	PTA-2074 06/09/00	Uni-ZAP XR	51	625	2	625	25	25	108	1	22	23	200

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
11	HHFKM76	PTA-2074 06/09/00	Uni-ZAP XR	52	2074	9	2060	32	32	109	1	22	23	100
12	HMALI42	PTA-2074 06/09/00	Uni-ZAP XR	22	785	12	554	187	187	79	1	24	25	113
13	HPJEV11	PTA-2074 06/09/00	Uni-ZAP XR	23	514	1	514	214	214	80	1	44	45	100
14	HTTKT43	PTA-2074 06/09/00	Uni-ZAP XR	24	2286	1	2286	30	30	81	1	45	46	168
14	HTTKT43	PTA-2074 06/09/00	Uni-ZAP XR	53	306	1	306	46	46	110	1	45	46	87
14	HTTKT43	PTA-2074 06/09/00	Uni-ZAP XR	54	278	1	265		177	111	1	1	2	49
15	HPJEE14	PTA-2074 06/09/00	Uni-ZAP XR	25	479	1	479	52	52	82	1	22	23	141
15	HPJEE14	PTA-2074 06/09/00	Uni-ZAP XR	55	547	1	547	45	45	112	1	22	23	141
16	HPJDA23	PTA-2074 06/09/00	Uni-ZAP XR	26	644	1	644	175	175	83	1	39	40	94
16	HPJDA23	PTA-2074 06/09/00	Uni-ZAP XR	56	387	1	387		364	113	1	1	2	49
17	HTPFX69	PTA-2074 06/09/00	Uni-ZAP XR	27	1803	1	1803	231	231	84	1	18	19	125

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
17	HTPFX69	PTA-2074 06/09/00	Uni-ZAP XR	57	1668	1	1668	128	128	114	1	19	20	125
17	HTPFX69	PTA-2074 06/09/00	Uni-ZAP XR	58	277	1	277	120	120	115	1	18	19	52
17	HTPFX69	PTA-2074 06/09/00	Uni-ZAP XR	59	3714	58	886		3684	116	1	1	2	1134
18	HTFOS57	PTA-2074 06/09/00	pSport1	28	1928	420	1928	633	633	85	1	27	28	211
18	HTFOS57	PTA-2074 06/09/00	pSport1	60	1039	427	1039	14	14	117	1	1	2	215
18	HTFOS57	PTA-2074 06/09/00	pSport1	61	678	2	678		255	118	1	1	2	69
19	HXOAC69	PTA-2074 06/09/00	pSport1	29	2045	1369	1916	1610	1610	86	1	21	22	98
19	HXOAC69	PTA-2074 06/09/00	pSport1	62	1406	728	1401	969	969	119	1	21	22	98
20	HWMAF61	PTA-2074 06/09/00	pSport1	30	2089	1	2089	173	173	87	1	23	24	547
20	HWMAF61	PTA-2074 06/09/00	pSport1	63	771	1	771	281	281	120	1			1
20	HWMAF61	PTA-2074 06/09/00	pSport1	64	1177	1	1177	173	173	121	1	23	24	303

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
20	HWMMAF61	PTA-2074 06/09/00	pSport1	65	684	1	684	110	110	122	1	23	24	191
20	HWMMAF61	PTA-2074 06/09/00	pSport1	66	771	1	771		771	123	1	1	2	165
21	HMSOC30	PTA-2074 06/09/00	Uni-ZAP XR	31	807	11	807	129	129	88	1	20	21	111
21	HMSOC30	PTA-2074 06/09/00	Uni-ZAP XR	67	822	1	822		758	124	1	1	2	148

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently

accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also
5 hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

10 Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the
15 actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the
20 generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods.
25 The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

30 The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed

herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

Table 2 provides predicted epitopes contained in certain embodiments of the invention and polynucleotide sequences that may be disclaimed according to certain embodiments of the invention. The first column refers to each "Gene #" described above in Table 1. The second column provides the sequence identifier, "NT SEQ ID NO:X", for polynucleotide sequences disclosed in Table 1. The third column provides the sequence identifier, "AA SEQ ID NO:Y", for polypeptide sequences disclosed in Table 1. The fourth column provides a unique integer "ntA" where "ntA" is any integer between 1 and the final nucleotide minus 15 of SEQ ID NO:X, and the fifth column provides a unique integer "ntB" where "ntB" is any integer between 15 and the final nucleotide of SEQ ID NO:X, where both ntA and ntB correspond to the positions of nucleotide residues shown in SEQ ID NO:X, and where ntB is greater than or equal to a + 14. For each of the polynucleotides shown as SEQ ID NO:X, the uniquely defined integers can be substituted into the general formula of a-b, and used to describe polynucleotides which may be preferably excluded from the invention. Column 6 lists residues comprising predicted epitopes contained in the polypeptides encoded by each of the preferred ORFs (SEQ ID NO:Y). Identification of potential immunogenic regions was performed according to the method of Jameson and Wolf ((1988) CABIOS, 4; 181-186); specifically, the Genetics Computer Group (GCG) implementation of this algorithm, embodied in the program PEPTIDESTRUCTURE (Wisconsin Package v10.0, Genetics Computer Group (GCG), Madison, Wisc.). This

method returns a measure of the probability that a given residue is found on the surface of the protein. Regions where the antigenic index score is greater than 0.9 over at least 6 amino acids are indicated in Table 2 as "Predicted epitopes".

Polypeptides of the invention may possess one, two, three, four, five or more
5 antigenic epitopes comprising residues described in Table 2. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant may vary slightly.

Table 3 summarizes the expression profile of polynucleotides corresponding to the clones disclosed in Table 1. The first column provides a unique clone
10 identifier, "Clone ID", for a cDNA clone related to each contig sequence disclosed in Table 1. Column 2, "Library Code(s)" shows the expression profile of tissue and/or cell line libraries which express the polynucleotides of the invention. Each Library Code in column 2 represents a tissue/cell source identifier code corresponding to the Library Code and Library description provided in Table 5. Expression of these
15 polynucleotides was not observed in the other tissues and/or cell libraries tested. One of skill in the art could routinely use this information to identify tissues which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue expression.

20 Table 4, column 1, provides a nucleotide sequence identifier, "SEQ ID NO:X," that matches a nucleotide SEQ ID NO:X disclosed in Table 1, column 5. Table 4, column 2, provides the chromosomal location, "Cytologic Band or Chromosome," of polynucleotides corresponding to SEQ ID NO:X. Chromosomal location was determined by finding exact matches to EST and cDNA sequences
25 contained in the NCBI (National Center for Biotechnology Information) UniGene database. Given a presumptive chromosomal location, disease locus association was determined by comparison with the Morbid Map, derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD)
30 and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>). If the putative chromosomal location of the Query overlapped with the chromosomal

location of a Morbid Map entry, the OMIM reference identification number of the morbid map entry is provided in Table 4, column 3, labelled "OMIM Reference(s)." A key to the OMIM reference identification numbers is provided in Table 6.

5 Table 5 provides a key to the Library Code disclosed in Table 3. Column 1 provides the Library Code disclosed in Table 3, column 2. Column 2 provides a description of the tissue or cell source from which the corresponding library was derived. Library codes corresponding to diseased Tissues are indicated in column 3 with the word "disease".

10 Table 6 provides a key to the OMIM reference identification numbers disclosed in Table 4, column 3. OMIM reference identification numbers (Column 1) were derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine, (Bethesda, MD) 2000. World Wide Web
15 URL: <http://www.ncbi.nlm.nih.gov/omim/>). Column 2 provides diseases associated with the cytologic band disclosed in Table 4, column 2, as determined using the Morbid Map database.

Table 2

Gene #	NT SEQ ID NO: X	AA SEQ ID NO: Y	nt A	nt B	Predicted Epitopes
1	11	68	1 - 2458	15 - 2472	Pro-104 to Pro-120.
1	32	89	1 - 419	15 - 433	
1	33	90	1 - 995	15 - 1009	Asn-1 to Ser-8 His-13 to Leu-20.
2	12	69	1 - 4023	15 - 4037	Arg-92 to Glu-110 Gly-148 to Arg-159.
2	34	91	1 - 669	15 - 683	Arg-92 to Glu-110.
2	35	92	1 - 3071	15 - 3085	Pro-76 to Thr-81.
3	13	70	1 - 1579	15 - 1593	
3	36	93	1 - 759	15 - 773	Arg-6 to Trp-11.
4	14	71	1 - 2938	15 - 2952	Asp-4 to Trp-9 Thr-24 to Arg-30 Pro-65 to Glu-70 Ser-97 to Glu-112 Gln-150 to Pro-156 Cys-184 to Phe-190 Leu-211 to Arg-224 Gln-266 to His-271 Gly-313 to Gly-320 Asp-397 to Glu-402 Pro-408 to Gly-414 Lys-426 to Asn-432 Pro-470 to Arg-477 Pro-479 to His-484.
4	37	94	1 - 3033	15 - 3047	Asp-4 to Trp-9 Thr-24 to Arg-30 Pro-65 to Glu-70 Ser-97 to Glu-112 Gln-150 to Pro-156.
4	38	95	1 - 1823	15 - 1837	Asp-4 to Trp-9 Thr-24 to Arg-30 Pro-65 to Glu-70 Ser-97 to Glu-112 Gln-150 to Pro-156.
4	39	96	1 - 590	15 - 604	Pro-5 to Arg-10 Ala-14 to Gly-24 Leu-41 to Arg-54 Ser-56 to Pro-63 Leu-94 to Arg-99 Ala-104 to Pro-121.
5	15	72	1 - 1339	15 - 1353	Pro-67 to Arg-78 Glu-152 to Arg-159 Ser-193 to Leu-200 Asp-210 to Lys-223.
5	40	97	1 - 1299	15 - 1313	Pro-67 to Arg-78 Glu-152 to Arg-159 Ser-193 to Leu-200 Asp-210 to Lys-223.
5	41	98	1 - 1338	15 - 1352	Pro-67 to Arg-78.
6	16	73	1 - 1778	15 - 1792	Ala-28 to His-41 Pro-43 to Lys-60.

6	42	99	1 - 1669	15 - 1683	Ala-28 to His-41 Pro-43 to Lys-60.
6	43	100	1 - 968	15 - 982	Ser-46 to Leu-53 Leu-61 to Arg-66 Leu-101 to Tyr-109 Cys-112 to Glu-119 Lys-124 to Thr-131.
7	17	74	1 - 655	15 - 669	Leu-63 to Gly-73.
7	44	101	1 - 516	15 - 530	Leu-63 to Gly-73.
8	18	75	1 - 3800	15 - 3814	Met-1 to Tyr-7.
8	45	102	1 - 527	15 - 541	Met-1 to Tyr-7.
8	46	103	1 - 2169	15 - 2183	Asn-77 to Met-83.
9	19	76	1 - 2823	15 - 2837	Arg-36 to Asn-48 Pro-60 to Gly-70 Arg-85 to Ser-94 Pro-96 to Ser-114 His-151 to Ser-169 Lys-183 to Pro-204 Pro-249 to Asn-258 Glu-265 to Ser-294 Phe-298 to Thr-313 Ser-331 to Gly-352 His-373 to Cys-381 Ser-389 to Lys-396 Glu-403 to Phe-413.
9	47	104	1 - 2823	15 - 2837	Arg-36 to Asn-48 Pro-60 to Gly-70 Arg-85 to Ser-94 Pro-96 to Ser-114 His-151 to Ser-169 Lys-183 to Pro-204.
9	48	105	1 - 2704	15 - 2718	Arg-36 to Asn-48 Pro-60 to Gly-70 Arg-85 to Ser-94 Pro-96 to Ser-114 Thr-159 to Gln-165 Pro-174 to Gln-179.
10	20	77	1 - 1491	15 - 1505	Arg-43 to Cys-48.
10	49	106	1 - 557	15 - 571	Arg-43 to Cys-48.
10	50	107	1 - 807	15 - 821	Lys-23 to Leu-33 Asp-40 to Cys-57.
11	21	78	1 - 1494	15 - 1508	Arg-29 to Arg-35 Cys-64 to Thr-73 Thr-81 to Glu-87 Lys-123 to Met-129 Lys-179 to Val-189 Ser-237 to Gln-250 Ser-264 to Lys-271 His-294 to Gln-300 Ser-346 to Thr-351.
11	51	108	1 - 611	15 - 625	Arg-29 to Arg-35 Cys-64 to Thr-73 Thr-81 to Glu-87 Lys-123 to Met-129.
11	52	109	1 - 2060	15 - 2074	
12	22	79	1 - 771	15 - 785	Ser-2 to Trp-7

					Gln-44 to Lys-53 Ser-80 to Gly-88.
13	23	80	1 - 500	15 - 514	
14	24	81	1 - 2272	15 - 2286	Glu-16 to Pro-24.
14	53	110	1 - 292	15 - 306	Glu-16 to Pro-24.
14	54	111	1 - 264	15 - 278	
15	25	82	1 - 465	15 - 479	Trp-21 to Cys-38 Gln-77 to Trp-85.
15	55	112	1 - 533	15 - 547	Trp-21 to Cys-38 Gln-77 to Trp-85.
16	26	83	1 - 630	15 - 644	Ser-18 to Arg-25.
16	56	113	1 - 373	15 - 387	
17	27	84	1 - 1789	15 - 1803	
17	57	114	1 - 1654	15 - 1668	
17	58	115	1 - 263	15 - 277	Ser-39 to Leu-45.
17	59	116	1 - 3700	15 - 3714	Pro-5 to Ser-12 Arg-20 to Lys-35 Gly-45 to Gly-56 Pro-81 to Cys-99 Arg-195 to Ser-207 Ser-287 to Pro-292 Thr-301 to Gly-306 Asn-336 to Met-341 Ser-357 to Arg-364 Cys-416 to Asp-430 Gln-450 to Asp-455 Pro-509 to Thr-516 Arg-523 to Gly-529 Lys-541 to Lys-546 Ser-623 to Arg-634 Tyr-636 to Gln-642 Asp-661 to Leu-671 Glu-692 to Lys-703 Gln-734 to Tyr-742 Thr-792 to Ile-798 Pro-806 to Thr-811 Phe-875 to Gly-880 Ser-886 to Gln-891 Val-897 to Glu-903 Glu-926 to Asn-932 Arg-945 to Asp-950 Pro-952 to Lys-958 Gly-991 to Gly-1001 Pro-1019 to Trp-1028 Lys-1082 to Met-1091 Val-1098 to Gly-1104 Cys-1116 to Glu-1122.
18	28	85	1 - 1914	15 - 1928	
18	60	117	1 - 1025	15 - 1039	Leu-31 to Glu-39 Gly-56 to Gln-64 Asn-109 to Thr-115.
18	61	118	1 - 664	15 - 678	Lys-6 to Thr-25.
19	29	86	1 - 2031	15 - 2045	Glu-34 to Gly-41 Ile-43 to Gly-62 Pro-85 to Ser-92.
19	62	119	1 - 1392	15 - 1406	Glu-34 to Gly-41

					Ile-43 to Gly-62 Pro-85 to Ser-92.
20	30	87	1 - 2075	15 - 2089	Tyr-46 to Ala-51 Leu-80 to Phe-89 Pro-108 to Asp-116 His-127 to Gly-136 Ala-195 to Asp-201 Asp-235 to Glu-240 Asn-296 to Ile-303 Trp-398 to Tyr-403 Leu-426 to Asn-431.
20	63	120	1 - 757	15 - 771	
20	64	121	1 - 1163	15 - 1177	Tyr-46 to Ala-51 Leu-80 to Phe-89 Pro-108 to Asp-116 His-127 to Gly-136.
20	65	122	1 - 670	15 - 684	Tyr-46 to Ala-51 Leu-80 to Phe-89 Pro-108 to Asp-116 His-127 to Gly-136.
20	66	123	1 - 757	15 - 771	
21	31	88	1 - 793	15 - 807	Leu-24 to Ser-30.
21	67	124	1 - 808	15 - 822	

Table 3

Clone ID	Library Code(s)
HFKLX38	H0620
HUVFH14	H0030 H0031 H0046 H0144 H0156 H0413 H0510 H0553 H0574 H0575 H0586 H0587 H0623 H0644 H0658 H0707 L1290 S0003 S0206 S0330 S0444
HWMH36	H0522 L1290
HUVHI35	H0116 H0213 H0252 H0266 H0286 H0288 H0305 H0306 H0370 H0412 H0433 H0435 H0521 H0522 H0529 H0539 H0542 H0551 H0555 H0559 H0560 H0590 H0619 H0620 H0623 H0652 H0656 H0673 L1290 S0001 S0002 S0011 S0031 S0040 S0046 S0126 S0132 S0152 S0342 S0344 S0360 S0418 S0446 S3014 T0049
HHFML08	H0100 H0619 L1290 S0216
HE2KK74	H0586 H0624 L1290 S0360 T0041
HFKME15	H0012 H0620
HE2LW65	H0013 H0030 H0032 H0038 H0040 H0068 H0085 H0144 H0171 H0212 H0251 H0268 H0351 H0369 H0393 H0412 H0413 H0435 H0444 H0486 H0519 H0520 H0529 H0542 H0544 H0545 H0546 H0575 H0591 H0624 H0628 H0644 H0645 H0648 H0658 H0665 H0670 H0672 L1290 S0003 S0007 S0026 S0027 S0031 S0044 S0045 S0046 S0051 S0126 S0132 S0210 S0282 S0354 S0358 S0360 S0374 S0410 S0420 S0438 S0442 S0468 S0472 T0010
HTPHS66	H0013 H0040 H0057 H0090 H0170 H0328 H0478 H0539 H0544 H0545 H0616 H0620 H0622 H0624 H0696 L1290 S0126 S0192 S0194 S0212 T0039 T0060
HPJEG57	S0152
HHFKM76	H0031 H0046 H0052 H0063 H0081 H0086 H0163 H0178 H0309 H0427 H0457 H0497 H0510 H0519 H0521 H0522 H0547 H0550 H0551 H0553 H0560 H0563 H0587 H0617 H0619 H0620 L0022 L1290 S0010 S0028 S0036 S0046 S0152 S0278 S0360 S0388 S0392 S0404 S0440
HMALI42	H0521 L0022 S0002 S0142 S0144 S0278 S0344
HPJEV11	H0370 H0438 S0152 S0354
HTTKT43	H0009 H0012 H0013 H0039 H0040 H0050 H0051 H0052 H0069 H0082 H0083 H0090 H0100 H0135 H0144 H0179 H0194 H0212 H0264 H0265 H0271 H0286 H0295 H0309 H0310 H0318 H0327 H0328 H0341 H0352 H0370 H0394 H0422 H0423 H0445 H0483 H0486 H0492 H0497 H0518 H0520 H0521 H0529 H0543 H0547 H0553 H0555 H0556 H0575 H0576 H0596 H0599 H0600 H0616 H0619 H0620 H0622 H0628 H0634 H0650 H0659 H0663 H0670 H0685 H0687 L0022 S0002 S0022 S0027 S0028 S0045 S0134 S0150 S0192 S0194 S0206 S0210 S0212 S0222 S0326 S0356 S0372 S0388 S0424 T0042 T0115
HPJEE14	S0152
HPJDA23	H0013 H0038 H0052 H0194 L0022 S0021 S0152 T0006 T0082
HTPFX69	H0038 H0039 H0050 H0051 H0057 H0059 H0083 H0085 H0086 H0087 H0100 H0135 H0136 H0144 H0169 H0176 H0178 H0181 H0188 H0196 H0208 H0213 H0250 H0293 H0318 H0333 H0351 H0352 H0370 H0393 H0395 H0405 H0412 H0413 H0423 H0424 H0428 H0445 H0483 H0484 H0520 H0521 H0529 H0543 H0544 H0545 H0546 H0547 H0549 H0556 H0581 H0591 H0593 H0594 H0596 H0597 H0604 H0617 H0618 H0620 H0622 H0635 H0641 H0648 H0657 H0659 H0660 H0661 H0672 H0674 H0683 H0684 H0689 H0690 H0694 L0022 N0006 S0007 S0010 S0011 S0026 S0028 S0037 S0045 S0051 S0106 S0142 S0276 S0278 S0360 S0418 S0420 S0422 S3014 T0004 T0006 T0010 T0039 T0041 T0048 T0049 T0067 T0069
HTFOS57	H0428 H0494 H0521 H0645 L0022 S0424

HXOAC69	H0135 H0252 H0253 H0255 H0261 H0264 H0271 H0318 H0333 H0392 H0402 H0421 H0455 H0575 H0581 H0587 H0618 H0624 H0625 H0664 L0022 S0052 S0110 S0116 S0222 S0360 S0428 S0466
HWMAF61	H0506 L0022 S0354
HMSOC30	H0008 H0031 H0179 H0265 H0266 H0271 H0288 H0306 H0435 H0441 H0618 L0022

Table 4

SEQ ID NO: X	Cytologic Band or Chromosome:	OMIM Reference(s):
13	16	

Table 5

Library Code	Library Description	Disease
H0008	Whole 6 Week Old Embryo	
H0009	Human Fetal Brain	
H0012	Human Fetal Kidney	
H0013	Human 8 Week Whole Embryo	
H0030	Human Placenta	
H0031	Human Placenta	
H0032	Human Prostate	
H0038	Human Testes	
H0039	Human Pancreas Tumor	disease
H0040	Human Testes Tumor	disease
H0046	Human Endometrial Tumor	disease
H0050	Human Fetal Heart	
H0051	Human Hippocampus	
H0052	Human Cerebellum	
H0057	Human Fetal Spleen	
H0059	Human Uterine Cancer	disease
H0063	Human Thymus	
H0068	Human Skin Tumor	disease
H0069	Human Activated T-Cells	
H0081	Human Fetal Epithelium (Skin)	
H0082	Human Fetal Muscle	
H0083	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	
H0085	Human Colon	
H0086	Human epithelioid sarcoma	disease
H0087	Human Thymus	
H0090	Human T-Cell Lymphoma	disease
H0100	Human Whole Six Week Old Embryo	
H0116	Human Thymus Tumor, subtracted	
H0135	Human Synovial Sarcoma	
H0136	Supt Cells, cyclohexamide treated	
H0144	Nine Week Old Early Stage Human	
H0156	Human Adrenal Gland Tumor	disease
H0163	Human Synovium	
H0169	Human Prostate Cancer, Stage C fraction	disease
H0170	12 Week Old Early Stage Human	
H0171	12 Week Old Early Stage Human, II	
H0176	CAMA1Ee Cell Line	
H0178	Human Fetal Brain	
H0179	Human Neutrophil	
H0181	Human Primary Breast Cancer	disease
H0188	Human Normal Breast	
H0194	Human Cerebellum, subtracted	
H0196	Human Cardiomyopathy, subtracted	
H0208	Early Stage Human Lung, subtracted	
H0212	Human Prostate, subtracted	
H0213	Human Pituitary, subtracted	
H0250	Human Activated Monocytes	
H0251	Human Chondrosarcoma	disease
H0252	Human Osteosarcoma	disease

H0253	Human adult testis, large inserts	
H0255	breast lymph node CDNA library	
H0261	H. cerebellum, Enzyme subtracted	
H0264	human tonsils	
H0265	Activated T-Cell (12hs)/Thiouridine labelledEco	
H0266	Human Microvascular Endothelial Cells, fract. A	
H0268	Human Umbilical Vein Endothelial Cells, fract. A	
H0271	Human Neutrophil, Activated	
H0286	Human OB MG63 treated (10 nM E2) fraction I	
H0288	Human OB HOS control fraction I	
H0293	WI 38 cells	
H0295	Amniotic Cells - Primary Culture	
H0305	CD34 positive cells (Cord Blood)	
H0306	CD34 depleted Buffy Coat (Cord Blood)	
H0309	Human Chronic Synovitis	disease
H0310	human caudate nucleus	
H0318	HUMAN B CELL LYMPHOMA	disease
H0327	human corpus colosum	
H0328	human ovarian cancer	disease
H0333	Hemangiopericytoma	disease
H0341	Bone Marrow Cell Line (RS4,11)	
H0351	Glioblastoma	disease
H0352	wilm's tumor	disease
H0369	H. Atrophic Endometrium	
H0370	H. Lymph node breast Cancer	disease
H0392	H. Meningima, M1	
H0393	Fetal Liver, subtraction II	
H0394	A-14 cell line	
H0395	A1-CELL LINE	
H0402	CD34 depleted Buffy Coat (Cord Blood), re-excision	
H0405	Human Pituitary, subtracted VI	
H0412	Human umbilical vein endothelial cells, IL-4 induced	
H0413	Human Umbilical Vein Endothelial Cells, uninduced	
H0421	Human Bone Marrow, re-excision	
H0422	T-Cell PHA 16 hrs	
H0423	T-Cell PHA 24 hrs	
H0424	Human Pituitary, subt IX	
H0427	Human Adipose	
H0428	Human Ovary	
H0433	Human Umbilical Vein Endothelial cells, frac B, re-excision	
H0435	Ovarian Tumor 10-3-95	
H0438	H. Whole Brain #2, re-excision	
H0441	H. Kidney Cortex, subtracted	
H0444	Spleen metastatic melanoma	disease
H0445	Spleen, Chronic lymphocytic leukemia	disease
H0455	H. Striatum Depression, subt	
H0457	Human Eosinophils	
H0478	Salivary Gland, Lib 2	
H0483	Breast Cancer cell line, MDA 36	
H0484	Breast Cancer Cell line, angiogenic	
H0486	Hodgkin's Lymphoma II	disease
H0492	HL-60, RA 4h, Subtracted	
H0494	Keratinocyte	

H0497	HEL cell line	
H0506	Ulcerative Colitis	
H0510	Human Liver, normal	
H0518	pBMC stimulated w/ poly I/C	
H0519	NTERA2, control	
H0520	NTERA2 + retinoic acid, 14 days	
H0521	Primary Dendritic Cells, lib 1	
H0522	Primary Dendritic cells, frac 2	
H0529	Myeloid Progenitor Cell Line	
H0539	Pancreas Islet Cell Tumor	disease
H0542	T Cell helper I	
H0543	T cell helper II	
H0544	Human endometrial stromal cells	
H0545	Human endometrial stromal cells-treated with progesterone	
H0546	Human endometrial stromal cells-treated with estradiol	
H0547	NTERA2 teratocarcinoma cell line+retinoic acid (14 days)	
H0549	H. Epididymus, caput & corpus	
H0550	H. Epididymus, cauda	
H0551	Human Thymus Stromal Cells	
H0553	Human Placenta	
H0555	Rejected Kidney, lib 4	disease
H0556	Activated T-cell(12h)/Thiouridine-re-excision	
H0559	HL-60, PMA 4H, re-excision	
H0560	KMH2	
H0563	Human Fetal Brain, normalized 50021F	
H0574	Hepatocellular Tumor, re-excision	disease
H0575	Human Adult Pulmonary, re-excision	
H0576	Resting T-Cell, re-excision	
H0581	Human Bone Marrow, treated	
H0586	Healing groin wound, 6.5 hours post incision	disease
H0587	Healing groin wound, 7.5 hours post incision	disease
H0590	Human adult small intestine, re-excision	
H0591	Human T-cell lymphoma, re-excision	disease
H0593	Olfactory epithelium, nasal cavity	
H0594	Human Lung Cancer, re-excision	disease
H0596	Human Colon Cancer, re-excision	
H0597	Human Colon, re-excision	
H0599	Human Adult Heart, re-excision	
H0600	Healing Abdomen wound, 70&90 min post incision	disease
H0604	Human Pituitary, re-excision	
H0616	Human Testes, Reexcision	
H0617	Human Primary Breast Cancer Reexcision	disease
H0618	Human Adult Testes, Large Inserts, Reexcision	
H0619	Fetal Heart	
H0620	Human Fetal Kidney, Reexcision	
H0622	Human Pancreas Tumor, Reexcision	disease
H0623	Human Umbilical Vein, Reexcision	
H0624	12 Week Early Stage Human II, Reexcision	
H0625	Ku 812F Basophils Line	
H0628	Human Pre-Differentiated Adipocytes	
H0634	Human Testes Tumor, re-excision	disease
H0635	Human Activated T-Cells, re-excision	
H0641	LPS activated derived dendritic cells	

H0644	Human Placenta (re-excision)	
H0645	Fetal Heart, re-excision	
H0648	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot	disease
H0650	B-Cells	
H0652	Lung, Normal: (4005313 B1)	
H0656	B-cells (unstimulated)	
H0657	B-cells (stimulated)	
H0658	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	disease
H0659	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	disease
H0660	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	disease
H0661	Breast, Cancer: (4004943 A5)	disease
H0663	Breast, Cancer: (4005522 A2)	disease
H0664	Breast, Cancer: (9806C012R)	disease
H0665	Stromal cells 3.88	
H0670	Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	
H0672	Ovary, Cancer: (4004576 A8)	
H0673	Human Prostate Cancer, Stage B2, re-excision	
H0674	Human Prostate Cancer, Stage C, re-excision	
H0683	Ovarian cancer, Serous Papillary Adenocarcinoma	
H0684	Ovarian cancer, Serous Papillary Adenocarcinoma	
H0685	Adenocarcinoma of Ovary, Human Cell Line, # OVCAR-3	
H0687	Human normal ovary(#9610G215)	
H0689	Ovarian Cancer	
H0690	Ovarian Cancer, # 9702G001	
H0694	Prostate cancer (adenocarcinoma)	
H0696	Prostate Adenocarcinoma	
H0707	Stomach Cancer(S007635)	
L0022	Soares fetal liver spleen 1NFLS	
L1290	Human fetal heart, Lambda ZAP Express	
N0006	Human Fetal Brain	
S0001	Brain frontal cortex	
S0002	Monocyte activated	
S0003	Human Osteoclastoma	disease
S0007	Early Stage Human Brain	
S0010	Human Amygdala	
S0011	STROMAL -OSTEOCLASTOMA	disease
S0021	Whole brain	
S0022	Human Osteoclastoma Stromal Cells - unamplified	
S0026	Stromal cell TF274	
S0027	Smooth muscle, serum treated	
S0028	Smooth muscle, control	
S0031	Spinal cord	
S0036	Human Substantia Nigra	
S0037	Smooth muscle, IL1b induced	
S0040	Adipocytes	
S0044	Prostate BPH	disease
S0045	Endothelial cells-control	
S0046	Endothelial-induced	
S0051	Human Hypothalamus, Schizophrenia	disease
S0052	neutrophils control	
S0106	STRIATUM DEPRESSION	disease

S0110	Brain Amygdala Depression	disease
S0116	Bone marrow	
S0126	Osteoblasts	
S0132	Epithelial-TNF α and INF induced	
S0134	Apoptotic T-cell	
S0142	Macrophage-oxLDL	
S0144	Macrophage (GM-CSF treated)	
S0150	LNCAP prostate cell line	
S0152	PC3 Prostate cell line	
S0192	Synovial Fibroblasts (control)	
S0194	Synovial hypoxia	
S0206	Smooth Muscle- HASTE normalized	
S0210	Mesangial cell, frac 2	
S0212	Bone Marrow Stromal Cell, untreated	
S0216	Neutrophils IL-1 and LPS induced	
S0222	H. Frontal cortex,epileptic,re-excision	disease
S0276	Synovial hypoxia-RSF subtracted	
S0278	H Macrophage (GM-CSF treated), re-excision	
S0282	Brain Frontal Cortex, re-excision	
S0326	Mammary Gland	
S0330	Palate normal	
S0342	Adipocytes,re-excision	
S0344	Macrophage-oxLDL, re-excision	
S0354	Colon Normal II	
S0356	Colon Carcinoma	disease
S0358	Colon Normal III	
S0360	Colon Tumor II	disease
S0372	Larynx carcinoma III	disease
S0374	Normal colon	
S0388	Human Hypothalamus,schizophrenia, re-excision	disease
S0392	Salivary Gland	
S0404	Rectum normal	
S0410	Colon, tumour	
S0418	CHME Cell Line,treated 5 hrs	
S0420	CHME Cell Line,untreated	
S0422	Mo7e Cell Line GM-CSF treated (1ng/ml)	
S0424	TF-1 Cell Line GM-CSF Treated	
S0428	Neutrophils control, re-excision	
S0438	Liver Normal Met5No	
S0440	Liver Tumour Met 5 Tu	
S0442	Colon Normal	
S0444	Colon Tumor	disease
S0446	Tongue Tumour	
S0466	Larynx Tumor	disease
S0468	Ea.hy.926 cell line	
S0472	Lung Mesothelium	
S3014	Smooth muscle, serum induced,re-exc	
T0004	Human White Fat	
T0006	Human Pineal Gland	
T0010	Human Infant Brain	
T0039	HSA 172 Cells	
T0041	Jurkat T-cell G1 phase	
T0042	Jurkat T-Cell, S phase	

T0048	Human Aortic Endothelium	
T0049	Aorta endothelial cells + TNF-a	
T0060	Human White Adipose	
T0067	Human Thyroid	
T0069	Human Uterus, normal	
T0082	Human Adult Retina	
T0115	Human Colon Carcinoma (HCC) cell line	

Table 6

OMIM Reference	Description
300047	Mental retardation, X-linked 20
300071	Night blindness, congenital stationary, type 2
300110	Night blindness, congenital stationary, X-linked incomplete, 300071
300600	Ocular albinism, Forsius-Eriksson type
301000	Thrombocytopenia, X-linked, 313900 Wiskott-Aldrich syndrome
301830	Arthrogryposis, X-linked (spinal muscular atrophy, infantile, X-linked)
309470	Mental retardation, X-linked, syndromic-3, with spastic diplegia
309500	Renpenning syndrome-1
309610	Mental retardation, X-linked, syndromic-2, with dysmorphism and cerebral atrophy
309850	Brunner syndrome
311050	Optic atrophy, X-linked
312060	Properdin deficiency, X-linked

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides
5 are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification ,
10 such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially
15 purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

20 The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides
25 encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

Signal Sequences

30 The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature

forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, *Virus Res.* 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra.*) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., *Protein Engineering* 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty.

Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the

nucleotide coding sequence contained in a deposited cDNA clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein).

Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined

using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

10 If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

25 For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base

subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1,

Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal

ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding
5 regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or
10 added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of
15 several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

20 Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988
25 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

30 Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational

analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule
5 could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or
10 C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular
15 polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions,
20 inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

25 The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these
30 positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used.

5 (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification or (v) fusion of the polypeptide with another compound, such as albumin (including, but not limited to, recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt,

and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40,

41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel

chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

5 In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope
10 of the invention.

Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID
15 NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses
20 polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower
25 stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide
30 encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies

described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays
5 described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985),
10 further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30
15 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies,
20 that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

25 Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any
30 combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to

an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very
5 least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-
10 2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS),
15 while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an
20 immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the
25 peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to heterologous polypeptide sequences. For example,
30 polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof,

resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide). Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can

also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines
5 (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are
10 loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to
15 modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol.
20 Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA
25 segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections,
30 parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from

human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific,
5 trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol.
10 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be
15 specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

20 Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at
25 least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less
30 than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present

invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity

or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent
5 ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but
10 do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists
15 for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998);
20 Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the
30 antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g.,

Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and

potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the

hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be
5 produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated
10 using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an
15 antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage
20 gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280
25 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by
30 reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies,

including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using
5 methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and
10 antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule
15 in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol.*
20 *Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule.
25 Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence
30 comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be

humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska. et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation.

Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, J. *Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

30

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that
5 encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the
10 nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then
15 amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized
20 or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe
25 specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the
30 antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for

example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their
5 entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the
10 art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally
15 occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid
20 substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other
25 alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes
30 from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are

derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain
5 antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the
10 assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the
15 art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an
20 expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using
25 techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include,
30 for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or

light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the

vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary
5 cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously
10 selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression
15 vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign
20 polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the
25 GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in
Spodoptera frugiperda cells. The antibody coding sequence may be cloned
individually into non-essential regions (for example the polyhedrin gene) of the virus
30 and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp^rt- or ap^rt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hyg^r, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993);

Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

5 The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in
10 culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

 The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second
15 vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic
20 free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

 Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any
25 method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or
30 fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991);

Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide,
5 polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins
10 consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in
15 binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been
20 expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58
25 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA,
30 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags

useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof
5 conjugated to a diagnostic or therapeutic agent. The antibodies can be used
diagnostically to, for example, monitor the development or progression of a tumor as
part of a clinical testing procedure to, e.g., determine the efficacy of a given
treatment regimen. Detection can be facilitated by coupling the antibody to a
detectable substance. Examples of detectable substances include various enzymes,
10 prosthetic groups, fluorescent materials, luminescent materials, bioluminescent
materials, radioactive materials, positron emitting metals using various positron
emission tomographies, and nonradioactive paramagnetic metal ions. The detectable
substance may be coupled or conjugated either directly to the antibody (or fragment
thereof) or indirectly, through an intermediate (such as, for example, a linker known
15 in the art) using techniques known in the art. See, for example, U.S. Patent No.
4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics
according to the present invention. Examples of suitable enzymes include horseradish
peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase;
examples of suitable prosthetic group complexes include streptavidin/biotin and
20 avidin/biotin; examples of suitable fluorescent materials include umbelliferone,
fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine
fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material
includes luminol; examples of bioluminescent materials include luciferase, luciferin,
and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{111}In
25 or ^{99}Tc .

Further, an antibody or fragment thereof may be conjugated to a therapeutic
moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or
a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi . A cytotoxin
or cytotoxic agent includes any agent that is detrimental to cells. Examples include
30 paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin,
etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin,
dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-

dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α , TNF- β , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld

et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And
5 Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol.
10 Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it,
15 administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of
20 cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular
25 populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

30 These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to

prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

5 *Assays For Antibody Binding*

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent
10 assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular
15 Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium
20 deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or
25 more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing
30 the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the

antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC).
Some of these approaches are described in more detail below. Armed with the
teachings provided herein, one of ordinary skill in the art will know how to use the
antibodies of the present invention for diagnostic, monitoring or therapeutic purposes
5 without undue experimentation.

The antibodies of this invention may be advantageously utilized in
combination with other monoclonal or chimeric antibodies, or with lymphokines or
hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which
serve to increase the number or activity of effector cells which interact with the
10 antibodies.

The antibodies of the invention may be administered alone or in combination
with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal
therapy, immunotherapy and anti-tumor agents). Generally, administration of
products of a species origin or species reactivity (in the case of antibodies) that is the
15 same species as that of the patient is preferred. Thus, in a preferred embodiment,
human antibodies, fragments derivatives, analogs, or nucleic acids, are administered
to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or
neutralizing antibodies against polypeptides or polynucleotides of the present
20 invention, fragments or regions thereof, for both immunoassays directed to and
therapy of disorders related to polynucleotides or polypeptides, including fragments
thereof, of the present invention. Such antibodies, fragments, or regions, will
preferably have an affinity for polynucleotides or polypeptides of the invention,
including fragments thereof. Preferred binding affinities include those with a
25 dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M,
 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M,
 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

30 *Gene Therapy*

In a specific embodiment, nucleic acids comprising sequences encoding
antibodies or functional derivatives thereof, are administered to treat, inhibit or

prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded
5 protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991);
10 Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and
15 Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains
20 thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a
25 desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy
30 and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or

indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered
5 in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct
10 injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see,
15 e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for
20 cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al.,
25 Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral
30 genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can

be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be

carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted.

10 The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

25 In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of

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the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

5 In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Demonstration of Therapeutic or Prophylactic Activity

10 The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect
15 of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient
20 tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by
25 administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses,
30 chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above;

additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein

and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, 5 *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and 10 Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose 15 (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic 20 acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating 25 with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

30 The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term

"pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry

lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval
5 by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically
10 bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body
15 fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder,
20 comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With
25 respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier
30 thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art

(e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody
5 assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or
10 disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval
15 following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject
20 has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging
25 system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor
30 imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging:

The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds.,
Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode
of administration, the time interval following the administration for permitting the
5 labeled molecule to preferentially concentrate at sites in the subject and for unbound
labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or
6 to 12 hours. In another embodiment the time interval following administration is 5
to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by
10 repeating the method for diagnosing the disease or disease, for example, one month
after initial diagnosis, six months after initial diagnosis, one year after initial
diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods
known in the art for in vivo scanning. These methods depend upon the type of label
15 used. Skilled artisans will be able to determine the appropriate method for detecting a
particular label. Methods and devices that may be used in the diagnostic methods of
the invention include, but are not limited to, computed tomography (CT), whole body
scan such as position emission tomography (PET), magnetic resonance imaging
(MRI), and sonography.

20 In a specific embodiment, the molecule is labeled with a radioisotope and is
detected in the patient using a radiation responsive surgical instrument (Thurston et
al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with
a fluorescent compound and is detected in the patient using a fluorescence responsive
scanning instrument. In another embodiment, the molecule is labeled with a positron
25 emitting metal and is detected in the patient using positron emission-tomography. In
yet another embodiment, the molecule is labeled with a paramagnetic label and is
detected in a patient using magnetic resonance imaging (MRI).

Kits

30 The present invention provides kits that can be used in the above methods. In
one embodiment, a kit comprises an antibody of the invention, preferably a purified
antibody, in one or more containers. In a specific embodiment, the kits of the present

invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

10 In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

20 In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

25 In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled

monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target

cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).) Polynucleotides comprising or alternatively consisting of nucleic acids which encode these fusion proteins are also encompassed by the invention.

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a

fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if
5 the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

10 Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available.
15 As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

20 Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of
25 the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

30 The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the

vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic
5 Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or
10 ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

15 Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant,
20 insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine
25 encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

30 In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A

main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

15 In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an

expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and
5 immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example,
10 techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650,
15 published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using
20 techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid
25 analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine,
30 norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, α -methyl amino

acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, *e.g.*, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used,

depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an
5 average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000,
10 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by
15 reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those
20 skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated
25 polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for
30 therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention

relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least
5 dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and
10 fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing
15 polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at
20 least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional
25 embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as,
30 for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed

when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides
5 of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally
10 occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence
15 contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from
20 another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627
25 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper
30 polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al.,

Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described
5 in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

10 Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by
15 reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations
20 proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers
25 of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues
30 located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely

modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, 5 techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic 10 engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are 15 generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by 20 reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by 25 reference in its entirety).

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes 30 known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers,

since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers
5 (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an
10 amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping
15 strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety)..

Precise chromosomal location of the polynucleotides can also be achieved
20 using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon
25 Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

The polynucleotides of the present invention would likewise be useful for
30 radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London

(1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

5 Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins
10 University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

 Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined.
15 First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide
20 and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

 Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using
25 polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

 Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the
30 present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression

level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test
5 subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand
10 containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present
15 invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the
20 polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard
25 being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

30 By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples

include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art.

5 Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a
10 "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying
15 disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or
20 according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain
25 components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and
30 tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide

backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined
5 with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ($T_{sub.m}$) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

10 The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic
15 leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often
20 associated with inappropriate activation of proto-oncogenes. (Gelman, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by
25 insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelman et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelman et al., supra) Indeed, the human counterparts of the oncogenes involved in
30 some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelman et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of
5 HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl.
10 Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

15 In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al.,
20 Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix -
25 see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while
30 antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information

disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective
5 gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute
10 biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of
15 "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions
20 of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from
25 extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or
30 surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR

Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be
5 used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the
10 present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a
15 specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

20

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a
25 biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay
30 (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and

technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo
5 imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for
10 the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or
15 intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc . The labeled antibody or antibody fragment will then
20 preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

25 Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is
30 indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for

detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

5 Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair
10 proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune
15 response to proliferative cells or tissues).

 Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can
20 activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

 At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also
25 be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations,

lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and
5 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3,
10 pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters
15 include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters,
20 such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the
25 polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver,
30 spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular,

fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph
5 fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated
10 cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20
15 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

20 The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the
25 procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

30 The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a
5 tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated
10 by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are
15 particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

20 Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc.
25 Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol,
30 phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP

starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC),
dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine
5 (DOPE) can be used in various combinations to make conventional liposomes, with or
without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be
prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas
into a sonication vial. The sample is placed under a vacuum pump overnight and is
hydrated the following day with deionized water. The sample is then sonicated for 2
10 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an
inverted cup (bath type) probe at the maximum setting while the bath is circulated at
15EC. Alternatively, negatively charged vesicles can be prepared without sonication
to produce multilamellar vesicles or by extrusion through nucleopore membranes to
produce unilamellar vesicles of discrete size. Other methods are known and available
15 to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar
vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred.
The various liposome-nucleic acid complexes are prepared using methods well known
in the art. See, e.g., Straubinger et al., *Methods of Immunology*, 101:512-527 (1983),
20 which is herein incorporated by reference. For example, MLVs containing nucleic
acid can be prepared by depositing a thin film of phospholipid on the walls of a glass
tube and subsequently hydrating with a solution of the material to be encapsulated.
SUVs are prepared by extended sonication of MLVs to produce a homogeneous
population of unilamellar liposomes. The material to be entrapped is added to a
25 suspension of preformed MLVs and then sonicated. When using liposomes containing
cationic lipids, the dried lipid film is resuspended in an appropriate solution such as
sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and
then the preformed liposomes are mixed directly with the DNA. The liposome and
DNA form a very stable complex due to binding of the positively charged liposomes
30 to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are
prepared by a number of methods, well known in the art. Commonly used methods
include Ca^{2+} -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta*,

394:483 (1975); Wilson et al., Cell , 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA , 76:145 (1979)); and
5 reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA , 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the
10 ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469
15 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

20 In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis
25 virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-
30 19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any

means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

5 The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

 In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with
10 polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA
15 into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been
20 demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science, 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA, 76:6606 (1979)).

 Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993);
25 Rosenfeld et al., Cell, 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature, 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and
30 constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other

varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

5 The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth
10 factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein.
15 Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

20 Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available
25 depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the
30 rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is

administered by direct injection into or locally within the area of arteries.

Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide
5 construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include
10 recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection,
15 aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present
20 invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

25 Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of
30 polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of

the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

5 **Biological Activities**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological
10 activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders associated with the following systems.

15

Immune Activity

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing
20 diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of
25 these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

30 In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an

immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing, and/or prognosing immunodeficiencies, including both congenital and acquired immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type), Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are treated, prevented, diagnosed, and/or prognosing using the polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof.

Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic

mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic aplasia, immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented, diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of

the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, diagnosed
5 and/or prognosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing
spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune
10 thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Scoenlein purpura),
autoimmunocytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

Additional disorders that are likely to have an autoimmune component that
15 may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid
syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing
polychondritis, rheumatic heart disease, neuritis, uveitis ophthalmia,
polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary
20 inflammation, autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus,
and autoimmune inflammatory eye disorders.

Additional disorders that are likely to have an autoimmune component that
may be treated, prevented, diagnosed and/or prognosed with the compositions of the
invention include, but are not limited to, scleroderma with anti-collagen antibodies
25 (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed
connective tissue disease (often characterized, e.g., by antibodies to extractable
nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by
nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell,
microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often
30 characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility
(often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often
characterized, e.g., by glomerular basement membrane antibodies or immune

complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic
5 drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional disorders that may have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized,
10 e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondria antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by
15 myocardial antibodies), cardiomyopathy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

20 In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed
25 using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred
30 embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

5 In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention

10 In preferred embodiments, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a immunosuppressive agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, prognosing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could
15 be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells, including but not limited to, leukopenia, neutropenia, anemia, and thrombocytopenia. Alternatively, Polynucleotides, polypeptides, antibodies, and/or
20 agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with an increase in certain (or many) types of hematopoietic cells, including but not limited to, histiocytosis.

25 Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, diagnosed and/or prognosed using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, prognose, and/or diagnose anaphylaxis, hypersensitivity to an antigenic
30 molecule, or blood group incompatibility.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, may be used to treat, prevent, diagnose and/or prognose

IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

5 Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention have uses in the diagnosis, prognosis, prevention, and/or treatment of inflammatory conditions. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells
10 involved in an inflammatory response, these molecules can be used to prevent and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, complement-mediated hyperacute rejection,
15 nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1.), respiratory disorders (e.g., asthma and allergy); gastrointestinal disorders (e.g., inflammatory bowel disease); cancers (e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (e.g., multiple sclerosis; ischemic brain injury and/or stroke, traumatic brain
20 injury, neurodegenerative disorders (e.g., Parkinson's disease and Alzheimer's disease); AIDS-related dementia; and prion disease); cardiovascular disorders (e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (e.g., hepatitis, rheumatoid arthritis, gout, trauma,
25 pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogenic transplant rejection).

 Because inflammation is a fundamental defense mechanism, inflammatory disorders can effect virtually any tissue of the body. Accordingly, polynucleotides,
30 polypeptides, and antibodies of the invention, as well as agonists or antagonists thereof, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adrenalitis, alveolitis, angiocholecystitis, appendicitis,

balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis, chorditis, cochitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myositis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis, peritendonitis, peritonitis, pharyngitis, phlebitis, poliomyelitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, spondylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

10 In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose, prevent, and/or treat organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in
15 this case, the foreign transplanted immune cells destroy the host tissues.

Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments, polypeptides,
20 antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

In other embodiments, polypeptides, antibodies, or polynucleotides of the
25 invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose, prevent, and/or treat immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, polyarteritis nodosa, and immune complex-induced vasculitis.

Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the
30 invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected,

and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of
5 application listing infectious agents, etc), without necessarily eliciting an immune response.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a vaccine adjuvant that enhances immune responsiveness to an antigen. In a specific embodiment,
10 polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance tumor-specific immune responses.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be
15 enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In
20 another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, respiratory syncytial virus, Dengue, rotavirus, Japanese B encephalitis, influenza A and B, parainfluenza, measles, cytomegalovirus, rabies, Junin, Chikungunya, Rift Valley Fever, herpes simplex, and
25 yellow fever.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as
30 an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune

response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B.

In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or
5 symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meisseria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

In another specific embodiment, polypeptides, antibodies, polynucleotides
10 and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an
15 adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria) or Leishmania.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat
20 infectious diseases including silicosis, sarcoidosis, and idiopathic pulmonary fibrosis; for example, by preventing the recruitment and activation of mononuclear phagocytes.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an antigen for the
25 generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

In one embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep,
30 dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and

immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B
5 cell responsiveness to pathogens.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an activator of T cells.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent that
10 elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to induce
15 higher affinity antibodies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to increase serum immunoglobulin concentrations.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to
20 accelerate recovery of immunocompromised individuals.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among aged populations and/or neonates.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to,
25 concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the
30 beginning of recovery of T-cell populations. In another specific embodiment,

compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS, bone
10 marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or
15 treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen
25 presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to direct
30 an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to
5 virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or
10 Common Variable Immunodeficiency.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, polypeptides, antibodies,
15 polynucleotides and/or agonists or antagonists of the present invention are used in the pretreatment of bone marrow samples prior to transplant.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a gene-based therapy for genetically inherited disorders resulting in immuno-
20 incompetence/immunodeficiency such as observed among SCID patients.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leishmania.

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used in one or more of the
30 applications described herein, as they may apply to veterinary medicine.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of

blocking various aspects of immune responses to foreign agents or self. Examples of diseases or conditions in which blocking of certain aspects of immune responses may be desired include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and
5 diseases/disorders associated with pathogens.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus
10 and multiple sclerosis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and
15 blocking sepsis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related
20 idiopathic monoclonal gammopathies, and plasmacytomas.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated
25 and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

The polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat idiopathic hyper-eosinophilic
30 syndrome by, for example, preventing eosinophil production and migration.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit complement mediated cell lysis.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

10 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed to treat adult respiratory distress syndrome (ARDS).

15 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, and/or stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

20 In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to diagnose, prognose, treat, and/or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne
25 infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media,
30 conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii. Other diseases and disorders that may be prevented, diagnosed, prognosed, and/or treated with polynucleotides or

polypeptides, and/or agonists of the present invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction anemia, thrombocytopenia, and hemoglobinuria.

5 In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

10 In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to diagnose, prognose, prevent, and/or treat cancers or neoplasms including immune cell or immune tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be prevented, diagnosed, or treated by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, acute
15 myelogenous leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, EBV-transformed diseases, and/or diseases and disorders described in the section entitled "Hyperproliferative Disorders" elsewhere herein.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of
25 decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete
30 splenectomy.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the polypeptides of

the present invention (e.g., Fc fusion protein; see, e.g., Example 9). Agonists of the invention include, for example, binding or stimulatory antibodies, and soluble forms of the polypeptides (e.g., Fc fusion proteins; see, e.g., Example 9). polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention
5 may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (including, but not limited to, those listed above, and also including transgenic
10 animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741).
15 Administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention to such animals is useful for the generation of monoclonal antibodies against the polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention in an organ system listed above.

20 **Blood-Related Disorders**

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hemostatic (the stopping of bleeding) or thrombolytic (clot dissolving) activity. For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, and/or agonists
25 or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies, hemophilia), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or
30 antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be

important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to prevent, diagnose, prognose, and/or treat thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to prevent, diagnose, prognose, and/or treat diseases and disorders of the blood and/or blood forming organs associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hematopoietic activity (the formation of blood cells). For example, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to increase the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis

and/or treatment of anemias and leukopenias described below. Alternatively, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to decrease the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g.,
5 basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets.. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of leukocytoses, such as, for example eosinophilia.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists
10 of the present invention may be used to prevent, treat, or diagnose blood dyscrasia.

Anemias are conditions in which the number of red blood cells or amount of hemoglobin (the protein that carries oxygen) in them is below normal. Anemia may be caused by excessive bleeding, decreased red blood cell production, or increased red blood cell destruction (hemolysis). The polynucleotides, polypeptides, antibodies,
15 and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias. Anemias that may be treated prevented or diagnosed by the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include iron deficiency anemia, hypochromic anemia, microcytic anemia, chlorosis, hereditary sideroblastic anemia, idiopathic
20 acquired sideroblastic anemia, red cell aplasia, megaloblastic anemia (e.g., pernicious anemia, (vitamin B12 deficiency) and folic acid deficiency anemia), aplastic anemia, hemolytic anemias (e.g., autoimmune hemolytic anemia, microangiopathic hemolytic anemia, and paroxysmal nocturnal hemoglobinuria). The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may
25 be useful in treating, preventing, and/or diagnosing anemias associated with diseases including but not limited to, anemias associated with systemic lupus erythematosus, cancers, lymphomas, chronic renal disease, and enlarged spleens. The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias
30 arising from drug treatments such as anemias associated with methyldopa, dapsone, and/or sulfadruugs. Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing,

and/or diagnosing anemias associated with abnormal red blood cell architecture including but not limited to, hereditary spherocytosis, hereditary elliptocytosis, glucose-6-phosphate dehydrogenase deficiency, and sickle cell anemia.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists
5 of the present invention may be useful in treating, preventing, and/or diagnosing hemoglobin abnormalities, (e.g., those associated with sickle cell anemia, hemoglobin C disease, hemoglobin S-C disease, and hemoglobin E disease). Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating
10 thalassemias, including, but not limited to major and minor forms of alpha-thalassemia and beta-thalassemia.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating bleeding disorders including, but not limited
15 to, thrombocytopenia (e.g., idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura), Von Willebrand's disease, hereditary platelet disorders (e.g., storage pool disease such as Chediak-Higashi and Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, thromboasthenia, and Bernard-Soulier syndrome), hemolytic-uremic syndrome, hemophelias such as hemophilia A or Factor
20 VII deficiency and Christmas disease or Factor IX deficiency, Hereditary Hemorrhagic Telangiectasia, also known as Rendu-Osler-Weber syndrome, allergic purpura (Henoch Schonlein purpura) and disseminated intravascular coagulation.

The effect of the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention on the clotting time of blood may be monitored
25 using any of the clotting tests known in the art including, but not limited to, whole blood partial thromboplastin time (PTT), the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the recalcified activated clotting time, or the Lee-White Clotting time.

Several diseases and a variety of drugs can cause platelet dysfunction. Thus, in
30 a specific embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating acquired platelet dysfunction such as platelet dysfunction

accompanying kidney failure, leukemia, multiple myeloma, cirrhosis of the liver, and systemic lupus erythematosus as well as platelet dysfunction associated with drug treatments, including treatment with aspirin, ticlopidine, nonsteroidal anti-inflammatory drugs (used for arthritis, pain, and sprains), and penicillin in high doses.

- 5 In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders characterized by or associated with increased or decreased numbers of white blood cells. Leukopenia occurs when the number of white blood cells decreases below normal. Leukopenias
- 10 include, but are not limited to, neutropenia and lymphocytopenia. An increase in the number of white blood cells compared to normal is known as leukocytosis. The body generates increased numbers of white blood cells during infection. Thus, leukocytosis may simply be a normal physiological parameter that reflects infection. Alternatively, leukocytosis may be an indicator of injury or other disease such as cancer.
- 15 Leukocytoses, include but are not limited to, eosinophilia, and accumulations of macrophages. In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukopenia. In other specific
- 20 embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukocytosis.

- Leukopenia may be a generalized decreased in all types of white blood cells, or may be a specific depletion of particular types of white blood cells. Thus, in specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists
- 25 or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating decreases in neutrophil numbers, known as neutropenia. Neutropenias that may be diagnosed, prognosed, prevented, and/or treated by the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, infantile genetic agranulocytosis,
- 30 familial neutropenia, cyclic neutropenia, neutropenias resulting from or associated with dietary deficiencies (e.g., vitamin B 12 deficiency or folic acid deficiency), neutropenias resulting from or associated with drug treatments (e.g., antibiotic

regimens such as penicillin treatment, sulfonamide treatment, anticoagulant treatment, anticonvulsant drugs, anti-thyroid drugs, and cancer chemotherapy), and neutropenias resulting from increased neutrophil destruction that may occur in association with some bacterial or viral infections, allergic disorders, autoimmune diseases, conditions
5 in which an individual has an enlarged spleen (e.g., Felty syndrome, malaria and sarcoidosis), and some drug treatment regimens.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating lymphocytopenias (decreased numbers of B and/or T lymphocytes),
10 including, but not limited lymphocytopenias resulting from or associated with stress, drug treatments (e.g., drug treatment with corticosteroids, cancer chemotherapies, and/or radiation therapies), AIDS infection and/or other diseases such as, for example, cancer, rheumatoid arthritis, systemic lupus erythematosus, chronic infections, some viral infections and/or hereditary disorders (e.g., DiGeorge syndrome, Wiskott-
15 Aldrich Syndrome, severe combined immunodeficiency, ataxia telangiectasia).

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with macrophage numbers and/or macrophage function including, but not limited to, Gaucher's disease, Niemann-Pick
20 disease, Letterer-Siwe disease and Hand-Schuller-Christian disease.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with eosinophil numbers and/or eosinophil function including, but not limited to,
25 idiopathic hypereosinophilic syndrome, eosinophilia-myalgia syndrome, and Hand-Schuller-Christian disease.

In yet another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukemias and lymphomas including, but not
30 limited to, acute lymphocytic (lymphoblastic) leukemia (ALL), acute myeloid (myelocytic, myelogenous, myeloblastic, or myelomonocytic) leukemia, chronic lymphocytic leukemia (e.g., B cell leukemias, T cell leukemias, Sezary syndrome, and

Hairy cell leukemia), chronic myelocytic (myeloid, myelogenous, or granulocytic) leukemia, Hodgkin's lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, and mycosis fungoides.

5 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders of plasma cells including, but not limited to, plasma cell dyscrasias, monoclonal gammaopathies, monoclonal gammopathies of undetermined significance, multiple myeloma, macroglobulinemia, Waldenstrom's macroglobulinemia, cryoglobulinemia, and
10 Raynaud's phenomenon.

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing myeloproliferative disorders, including but not limited to, polycythemia vera, relative polycythemia, secondary polycythemia, myelofibrosis,
15 acute myelofibrosis, agnogenic myelod metaplasia, thrombocythemia, (including both primary and secondary thrombocythemia) and chronic myelocytic leukemia.

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as a treatment prior to surgery, to increase blood cell production.

20 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to enhance the migration, phagocytosis, superoxide production, antibody dependent cellular cytotoxicity of neutrophils, eosinophils and macrophages.

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or
25 agonists or antagonists of the present invention may be useful as an agent to increase the number of stem cells in circulation prior to stem cells pheresis. In another specific embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase the number of stem cells in circulation prior to platelet pheresis.

30 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase cytokine production.

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in preventing, diagnosing, and/or treating primary hematopoietic disorders.

5 **Hyperproliferative Disorders**

In certain embodiments, polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder
10 through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating,
15 differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

20 Examples of hyperproliferative disorders that can be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and
25 peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: Acute
30 Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute

Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease,
 Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's
 Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue Sarcoma, AIDS-Related
 Lymphoma, AIDS-Related Malignancies, Anal Cancer, Astrocytoma, Bile Duct
 5 Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumors, Breast
 Cancer, Cancer of the Renal Pelvis and Ureter, Central Nervous System (Primary)
 Lymphoma, Central Nervous System Lymphoma, Cerebellar Astrocytoma, Cerebral
 Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer,
 Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia,
 10 Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Childhood
 Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Childhood Extracranial
 Germ Cell Tumors, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma,
 Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic
 Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma,
 15 Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood
 Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood Soft Tissue
 Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic
 Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Colon Cancer, Cutaneous
 T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer,
 20 Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related
 Tumors, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumor, Extragonadal
 Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast
 Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal
 Carcinoid Tumor, Gastrointestinal Tumors, Germ Cell Tumors, Gestational
 25 Trophoblastic Tumor, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular
 Cancer, Hodgkin's Disease, Hodgkin's Lymphoma, Hypergammaglobulinemia,
 Hypopharyngeal Cancer, Intestinal Cancers, Intraocular Melanoma, Islet Cell
 Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer,
 Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer,
 30 Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant
 Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma,
 Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary Squamous

Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's
5 Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary Metastatic Squamous Neck Cancer, Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma, Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic
10 Cancer, Paraproteinemias, Purpura, Parathyroid Cancer, Penile Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Ureter Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary
15 Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive Neuroectodermal and Pineal Tumors, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Transitional Renal Pelvis and Ureter Cancer, Trophoblastic Tumors, Ureter and Renal
20 Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, Wilms' Tumor, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

In another preferred embodiment, polynucleotides or polypeptides, or agonists
25 or antagonists of the present invention are used to diagnose, prognose, prevent, and/or treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders described above. Such uses are indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia,
30 metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W. B. Saunders Co., Philadelphia, pp. 68-79.)

Hyperplasia is a form of controlled cell proliferation, involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. Hyperplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, angiofollicular mediastinal lymph node hyperplasia, angiolymphoid hyperplasia with eosinophilia, atypical melanocytic hyperplasia, basal cell hyperplasia, benign giant lymph node hyperplasia, cementum hyperplasia, congenital adrenal hyperplasia, congenital sebaceous hyperplasia, cystic hyperplasia, cystic hyperplasia of the breast, denture hyperplasia, ductal hyperplasia, endometrial hyperplasia, fibromuscular hyperplasia, focal epithelial hyperplasia, gingival hyperplasia, inflammatory fibrous hyperplasia, inflammatory papillary hyperplasia, intravascular papillary endothelial hyperplasia, nodular hyperplasia of prostate, nodular regenerative hyperplasia, pseudoepitheliomatous hyperplasia, senile sebaceous hyperplasia, and verrucous hyperplasia.

Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, agnogenic myeloid metaplasia, apocrine metaplasia, atypical metaplasia, autoparenchymatous metaplasia, connective tissue metaplasia, epithelial metaplasia, intestinal metaplasia, metaplastic anemia, metaplastic ossification, metaplastic polyps, myeloid metaplasia, primary myeloid metaplasia, secondary myeloid metaplasia, squamous metaplasia, squamous metaplasia of amnion, and symptomatic myeloid metaplasia.

Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation. Dysplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, anhidrotic

ectodermal dysplasia, anterofacial dysplasia, asphyxiating thoracic dysplasia, atridigital dysplasia, bronchopulmonary dysplasia, cerebral dysplasia, cervical dysplasia, chondroectodermal dysplasia, cleidocranial dysplasia, congenital ectodermal dysplasia, craniodiaphysial dysplasia, craniocarpotarsal dysplasia, 5 craniometaphysial dysplasia, dentin dysplasia, diaphysial dysplasia, ectodermal dysplasia, enamel dysplasia, encephalo-ophthalmic dysplasia, dysplasia epiphysialis hemimelia, dysplasia epiphysialis multiplex, dysplasia epiphysialis punctata, epithelial dysplasia, faciodigitogenital dysplasia, familial fibrous dysplasia of jaws, familial white folded dysplasia, fibromuscular dysplasia, fibrous dysplasia of bone, 10 florid osseous dysplasia, hereditary renal-retinal dysplasia, hidrotic ectodermal dysplasia, hypohidrotic ectodermal dysplasia, lymphopenic thymic dysplasia, mammary dysplasia, mandibulofacial dysplasia, metaphysial dysplasia, Mondini dysplasia, monostotic fibrous dysplasia, mucoepithelial dysplasia, multiple epiphysial dysplasia, oculoauriculovertebral dysplasia, oculodentodigital dysplasia, 15 oculovertebral dysplasia, odontogenic dysplasia, ophthalmomandibulomelic dysplasia, periapical cemental dysplasia, polyostotic fibrous dysplasia, pseudoachondroplastic spondyloepiphysial dysplasia, retinal dysplasia, septo-optic dysplasia, spondyloepiphysial dysplasia, and ventriculoradial dysplasia.

Additional pre-neoplastic disorders which can be diagnosed, prognosed, 20 prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, benign dysproliferative disorders (e.g., benign tumors, fibrocystic conditions, tissue hypertrophy, intestinal polyps, colon polyps, and esophageal dysplasia), leukoplakia, keratoses, Bowen's disease, Farmer's Skin, solar cheilitis, and solar keratosis.

25 In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or prognose disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

30 In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat cancers and neoplasms, including,

but not limited to those described herein. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat acute myelogenous leukemia.

5 Additionally, polynucleotides, polypeptides, and/or agonists or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be diagnosed, prognosed, prevented, and/or treated
10 by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma,
15 lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related
20 glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

 In preferred embodiments, polynucleotides, polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metastasis
25 of cancers, in particular those listed above.

 Additional diseases or conditions associated with increased cell survival that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such
30 as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic

(granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, emangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include, but are not limited to, neoplasms located in the liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

Another preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention

inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for

polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use
5 of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts,
10 which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the
15 polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in
20 tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described
25 disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may
30 be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some

of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

5 In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

10 The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

 It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or
15 neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments
20 thereof. Preferred binding affinities include those with a dissociation constant or K_d less than $5 \times 10^{-6}M$, $10^{-6}M$, $5 \times 10^{-7}M$, $10^{-7}M$, $5 \times 10^{-8}M$, $10^{-8}M$, $5 \times 10^{-9}M$, $10^{-9}M$, $5 \times 10^{-10}M$, $10^{-10}M$, $5 \times 10^{-11}M$, $10^{-11}M$, $5 \times 10^{-12}M$, $10^{-12}M$, $5 \times 10^{-13}M$, $10^{-13}M$, $5 \times 10^{-14}M$, $10^{-14}M$, $5 \times 10^{-15}M$, and $10^{-15}M$.

 Moreover, polypeptides of the present invention are useful in inhibiting the
25 angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl
30 Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al.,

Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction
5 of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al.,
10 Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs
15 or adjuvants, such as apoptonin, galectins, thioredoxins, anti-inflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

20 Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4
25 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions
30 containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide

antibodies of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Renal Disorders

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose disorders of the renal system. Renal disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, kidney failure, nephritis, blood vessel disorders of kidney, metabolic and congenital kidney disorders, urinary disorders of the kidney, autoimmune disorders, sclerosis and necrosis, electrolyte imbalance, and kidney cancers.

Kidney diseases which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, acute kidney failure, chronic kidney failure, atheroembolic renal failure, end-stage renal disease, inflammatory diseases of the kidney (e.g., acute glomerulonephritis, postinfectious glomerulonephritis, rapidly progressive glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis, familial nephrotic syndrome, membranoproliferative glomerulonephritis I and II, mesangial proliferative glomerulonephritis, chronic glomerulonephritis, acute tubulointerstitial nephritis, chronic tubulointerstitial nephritis, acute post-streptococcal glomerulonephritis (PSGN), pyelonephritis, lupus nephritis, chronic nephritis, interstitial nephritis, and post-streptococcal glomerulonephritis), blood vessel disorders of the kidneys (e.g., kidney infarction, atheroembolic kidney disease, cortical necrosis, malignant nephrosclerosis, renal vein thrombosis, renal underperfusion, renal retinopathy, renal ischemia-reperfusion, renal

artery embolism, and renal artery stenosis), and kidney disorders resulting from urinary tract disease (e.g., pyelonephritis, hydronephrosis, urolithiasis (renal lithiasis, nephrolithiasis), reflux nephropathy, urinary tract infections, urinary retention, and acute or chronic unilateral obstructive uropathy.)

5 In addition, compositions of the invention can be used to diagnose, prognose, prevent, and/or treat metabolic and congenital disorders of the kidney (e.g., uremia, renal amyloidosis, renal osteodystrophy, renal tubular acidosis, renal glycosuria, nephrogenic diabetes insipidus, cystinuria, Fanconi's syndrome, renal fibrocystic osteosis (renal rickets), Hartnup disease, Bartter's syndrome, Liddle's syndrome,
10 polycystic kidney disease, medullary cystic disease, medullary sponge kidney, Alport's syndrome, nail-patella syndrome, congenital nephrotic syndrome, CRUSH syndrome, horseshoe kidney, diabetic nephropathy, nephrogenic diabetes insipidus, analgesic nephropathy, kidney stones, and membranous nephropathy), and autoimmune disorders of the kidney (e.g., systemic lupus erythematosus (SLE),
15 Goodpasture syndrome, IgA nephropathy, and IgM mesangial proliferative glomerulonephritis).

 Compositions of the invention can also be used to diagnose, prognose, prevent, and/or treat sclerotic or necrotic disorders of the kidney (e.g., glomerulosclerosis, diabetic nephropathy, focal segmental glomerulosclerosis
20 (FSGS), necrotizing glomerulonephritis, and renal papillary necrosis), cancers of the kidney (e.g., nephroma, hypernephroma, nephroblastoma, renal cell cancer, transitional cell cancer, renal adenocarcinoma, squamous cell cancer, and Wilm's tumor), and electrolyte imbalances (e.g., nephrocalcinosis, pyuria, edema, hydronephritis, proteinuria, hyponatremia, hypernatremia, hypokalemia,
25 hyperkalemia, hypocalcemia, hypercalcemia, hypophosphatemia, and hyperphosphatemia).

 Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle
30 accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the

art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

5 **Cardiovascular Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose cardiovascular disorders, including, but not limited to, peripheral artery disease, such as limb ischemia:

10 Cardiovascular disorders include, but are not limited to, cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include, but are not limited to, aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia,
15 patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

20 Cardiovascular disorders also include, but are not limited to, heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy,
25 right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar
30 Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include, but are not limited to, sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-

branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular
5 rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve diseases include, but are not limited to, aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse,
10 tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include, but are not limited to, alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular
15 stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include, but are not limited to, coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis,
20 coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomas, bacillary angiomas, Hippiel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive
25 diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena
30 cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include, but are not limited to, dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include, but are not limited to, arteriosclerosis,
5 intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include, but are not limited to, carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral
10 arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia,
15 vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include, but are not limited to, air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include, but are not limited to, coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery
20 thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemic disorders include, but are not limited to, cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes, but is not limited to, aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome,
25 mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous
30 injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or

topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

5

Respiratory Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be used to treat, prevent, diagnose, and/or prognose diseases and/or disorders of the respiratory system.

10

Diseases and disorders of the respiratory system include, but are not limited to, nasal vestibulitis, nonallergic rhinitis (e.g., acute rhinitis, chronic rhinitis, atrophic rhinitis, vasomotor rhinitis), nasal polyps, and sinusitis, juvenile angiofibromas, cancer of the nose and juvenile papillomas, vocal cord polyps, nodules (singer's nodules), contact ulcers, vocal cord paralysis, laryngoceles, pharyngitis (e.g., viral and
15 bacterial), tonsillitis, tonsillar cellulitis, parapharyngeal abscess, laryngitis, laryngoceles, and throat cancers (e.g., cancer of the nasopharynx, tonsil cancer, larynx cancer), lung cancer (e.g., squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma), allergic disorders (eosinophilic pneumonia, hypersensitivity pneumonitis (e.g., extrinsic allergic alveolitis, allergic
20 interstitial pneumonitis, organic dust pneumoconiosis, allergic bronchopulmonary aspergillosis, asthma, Wegener's granulomatosis (granulomatous vasculitis), Goodpasture's syndrome)), pneumonia (e.g., bacterial pneumonia (e.g., *Streptococcus pneumoniae* (pneumococcal pneumonia), *Staphylococcus aureus* (staphylococcal pneumonia), Gram-negative bacterial pneumonia (caused by, e.g., *Klebsiella* and
25 *Pseudomonas spp.*), *Mycoplasma pneumoniae* pneumonia, *Hemophilus influenzae* pneumonia, *Legionella pneumophila* (Legionnaires' disease), and *Chlamydia psittaci* (Psittacosis)), and viral pneumonia (e.g., influenza, chickenpox (varicella).

Additional diseases and disorders of the respiratory system include, but are not limited to bronchiolitis, polio (poliomyelitis), croup, respiratory syncytial viral
30 infection, mumps, erythema infectiosum (fifth disease), roseola infantum, progressive rubella panencephalitis, german measles, and subacute sclerosing panencephalitis), fungal pneumonia (e.g., Histoplasmosis, Coccidioidomycosis, Blastomycosis, fungal

infections in people with severely suppressed immune systems (e.g., cryptococcosis, caused by *Cryptococcus neoformans*; aspergillosis, caused by *Aspergillus spp.*; candidiasis, caused by *Candida*; and mucormycosis)), *Pneumocystis carinii* (pneumocystis pneumonia), atypical pneumonias (e.g., *Mycoplasma* and *Chlamydia* spp.), opportunistic infection pneumonia, nosocomial pneumonia, chemical pneumonitis, and aspiration pneumonia, pleural disorders (e.g., pleurisy, pleural effusion, and pneumothorax (e.g., simple spontaneous pneumothorax, complicated spontaneous pneumothorax, tension pneumothorax)), obstructive airway diseases (e.g., asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic or acute bronchitis), occupational lung diseases (e.g., silicosis, black lung (coal workers' pneumoconiosis), asbestosis, berylliosis, occupational asthma, byssinosis, and benign pneumoconioses), Infiltrative Lung Disease (e.g., pulmonary fibrosis (e.g., fibrosing alveolitis, usual interstitial pneumonia), idiopathic pulmonary fibrosis, desquamative interstitial pneumonia, lymphoid interstitial pneumonia, histiocytosis X (e.g., Letterer-Siwe disease, Hand-Schüller-Christian disease, eosinophilic granuloma), idiopathic pulmonary hemosiderosis, sarcoidosis and pulmonary alveolar proteinosis), Acute respiratory distress syndrome (also called, e.g., adult respiratory distress syndrome), edema, pulmonary embolism, bronchitis (e.g., viral, bacterial), bronchiectasis, atelectasis, lung abscess (caused by, e.g., *Staphylococcus aureus* or *Legionella pneumophila*), and cystic fibrosis.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization

including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and

Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists
5 may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in
10 treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular
15 glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations;
20 ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a
25 polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists of the invention are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This
30 therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress

(approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, 5 retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of 10 prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for 15 treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, 20 capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes 25 simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared 30 for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and

administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the

compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated, prevented, diagnosed, and/or prognosed with the the polynucleotides, polypeptides, agonists and/or antagonists of the invention include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals,

arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation

5 controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (*Rochela minalia quintosa*), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and

10 fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

15 Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present

20 invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat

25 tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized

30 during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl

complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten
5 oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its
10 hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and
15 sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan
20 Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin;
25 Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum;
30 alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrone (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-

316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

5 Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, diagnosed, and/or prognosed using polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors,
10 pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's
15 syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

20 In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or
25 antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic
30 lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and

carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, diagnosed, and/or prognosed using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to

stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical

5 wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications
10 associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the
15 present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular grafts, Blair-Brown
20 grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as agonists or
25 antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach,
30 small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes,

mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

5 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides,
10 as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat
15 glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides,
20 as well as agonists or antagonists of the present invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large
25 intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the
30 production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associate with the under expression.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neural Activity and Neurological Diseases

The polynucleotides, polypeptides and agonists or antagonists of the invention may be used for the diagnosis and/or treatment of diseases, disorders, damage or injury of the brain and/or nervous system. Nervous system disorders that can be
5 treated with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian
10 patients) according to the methods of the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including
15 lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of
20 the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, or syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to, degeneration
25 associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including, but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and
30 alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy),

systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human
5 immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In one embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects
10 of hypoxia. In a further preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat or prevent neural cell injury associated with cerebral hypoxia. In one non-exclusive aspect of this embodiment, the
15 polypeptides, polynucleotides, or agonists or antagonists of the invention, are used to treat or prevent neural cell injury associated with cerebral ischemia. In another non-exclusive aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with cerebral infarction.

20 In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a stroke. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a stroke.

25 In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a heart attack. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a heart attack.

30 The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of

limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture either in the presence or absence of hypoxia or hypoxic conditions; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, in Zhang *et al.*, *Proc Natl Acad Sci USA* 97:3637-42 (2000) or in Arakawa *et al.*, *J. Neurosci.*, 10:3507-15 (1990); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk *et al.*, *Exp. Neurol.*, 70:65-82 (1980), or Brown *et al.*, *Ann. Rev. Neurosci.*, 4:17-42 (1981); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include, but are not limited to, disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Further, polypeptides or polynucleotides of the invention may play a role in neuronal survival; synapse formation; conductance; neural differentiation, etc. Thus, compositions of the invention (including polynucleotides, polypeptides, and agonists

or antagonists) may be used to diagnose and/or treat or prevent diseases or disorders associated with these roles, including, but not limited to, learning and/or cognition disorders. The compositions of the invention may also be useful in the treatment or prevention of neurodegenerative disease states and/or behavioural disorders. Such

5 neurodegenerative disease states and/or behavioral disorders include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition,

10 compositions of the invention may also play a role in the treatment, prevention and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

Additionally, polypeptides, polynucleotides and/or agonists or antagonists of the invention, may be useful in protecting neural cells from diseases, damage,

15 disorders, or injury, associated with cerebrovascular disorders including, but not limited to, carotid artery diseases (e.g., carotid artery thrombosis, carotid stenosis, or Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis (e.g., carotid artery thrombosis, sinus

20 thrombosis, or Wallenberg's Syndrome), cerebral hemorrhage (e.g., epidural or subdural hematoma, or subarachnoid hemorrhage), cerebral infarction, cerebral ischemia (e.g., transient cerebral ischemia, Subclavian Steal Syndrome, or vertebrobasilar insufficiency), vascular dementia (e.g., multi-infarct), leukomalacia, periventricular, and vascular headache (e.g., cluster headache or migraines).

25 In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be

30 used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache and migraine.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral

encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis,
5 encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, and Hallervorden-
10 Spatz Syndrome.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral
15 malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine
20 Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, and cerebral malaria.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis
25 which includes lymphocytic choriomeningitis, Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis, Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome, Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uveomeningoencephalitic syndrome, myelitis such as
30 transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as

Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie), and cerebral toxoplasmosis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention

5 include central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral

10 sceloris which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue

15 Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's

20 Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon-Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucopolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndromé, Rett

25 Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta.

30 Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hereditary motor and sensory neuropathies which include Charcot-Marie

Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, 5 apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, 10 broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, 15 myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic 20 Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, 25 spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, 30 Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia,

- amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes
- 5 Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia, Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis,
- 10 Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, and Diabetic neuropathies such as diabetic foot.
- 15 Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial
- 20 neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies
- 25 which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

Endocrine Disorders

- Polynucleotides or polypeptides, or agonists or antagonists of the present
- 30 invention, may be used to treat, prevent, diagnose, and/or prognose disorders and/or diseases related to hormone imbalance, and/or disorders or diseases of the endocrine

system.

Hormones secreted by the glands of the endocrine system control physical growth, sexual function, metabolism, and other functions. Disorders may be classified in two ways: disturbances in the production of hormones, and the inability of tissues to respond to hormones. The etiology of these hormone imbalance or endocrine system diseases, disorders or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy, injury or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular disease or disorder related to the endocrine system and/or hormone imbalance.

Endocrine system and/or hormone imbalance and/or diseases encompass disorders of uterine motility including, but not limited to: complications with pregnancy and labor (e.g., pre-term labor, post-term pregnancy, spontaneous abortion, and slow or stopped labor); and disorders and/or diseases of the menstrual cycle (e.g., dysmenorrhea and endometriosis).

Endocrine system and/or hormone imbalance disorders and/or diseases include disorders and/or diseases of the pancreas, such as, for example, diabetes mellitus, diabetes insipidus, congenital pancreatic agenesis, pheochromocytoma--islet cell tumor syndrome; disorders and/or diseases of the adrenal glands such as, for example, Addison's Disease, corticosteroid deficiency, virilizing disease, hirsutism, Cushing's Syndrome, hyperaldosteronism, pheochromocytoma; disorders and/or diseases of the pituitary gland, such as, for example, hyperpituitarism, hypopituitarism, pituitary dwarfism, pituitary adenoma, panhypopituitarism, acromegaly, gigantism; disorders and/or diseases of the thyroid, including but not limited to, hyperthyroidism, hypothyroidism, Plummer's disease, Graves' disease (toxic diffuse goiter), toxic nodular goiter, thyroiditis (Hashimoto's thyroiditis, subacute granulomatous thyroiditis, and silent lymphocytic thyroiditis), Pendred's syndrome, myxedema, cretinism, thyrotoxicosis, thyroid hormone coupling defect, thymic aplasia, Hurthle cell tumours of the thyroid, thyroid cancer, thyroid carcinoma, Medullary thyroid carcinoma; disorders and/or diseases of the parathyroid, such as, for example, hyperparathyroidism, hypoparathyroidism; disorders and/or diseases of the hypothalamus.

In addition, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases of the testes or ovaries, including cancer. Other disorders and/or diseases of the testes or ovaries further include, for example, ovarian cancer, polycystic ovary syndrome, Klinefelter's syndrome,
5 vanishing testes syndrome (bilateral anorchia), congenital absence of Leydig's cells, cryptorchidism, Noonan's syndrome, myotonic dystrophy, capillary haemangioma of the testis (benign), neoplasias of the testis and neo-testis.

Moreover, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases such as, for example,
10 polyglandular deficiency syndromes, pheochromocytoma, neuroblastoma, multiple Endocrine neoplasia, and disorders and/or cancers of endocrine tissues.

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose, prognose, prevent, and/or treat endocrine diseases and/or disorders
15 associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1, column 8 (Tissue Distribution Library Code).

Reproductive System Disorders

20 The polynucleotides or polypeptides, or agonists or antagonists of the invention may be used for the diagnosis, treatment, or prevention of diseases and/or disorders of the reproductive system. Reproductive system disorders that can be treated by the compositions of the invention, include, but are not limited to, reproductive system injuries, infections, neoplastic disorders, congenital defects, and
25 diseases or disorders which result in infertility, complications with pregnancy, labor, or parturition, and postpartum difficulties.

Reproductive system disorders and/or diseases include diseases and/or disorders of the testes, including testicular atrophy, testicular feminization, cryptorchism (unilateral and bilateral), anorchia, ectopic testis, epididymitis and
30 orchitis (typically resulting from infections such as, for example, gonorrhea, mumps, tuberculosis, and syphilis), testicular torsion, vasitis nodosa, germ cell tumors (e.g., seminomas, embryonal cell carcinomas, teratocarcinomas, choriocarcinomas, yolk sac

tumors, and teratomas), stromal tumors (e.g., Leydig cell tumors), hydrocele, hematocele, varicocele, spermatocele, inguinal hernia, and disorders of sperm production (e.g., immotile cilia syndrome, aspermia, asthenozoospermia, azoospermia, oligospermia, and teratozoospermia).

5 Reproductive system disorders also include disorders of the prostate gland, such as acute non-bacterial prostatitis, chronic non-bacterial prostatitis, acute bacterial prostatitis, chronic bacterial prostatitis, prostatodystonia, prostatosis, granulomatous prostatitis, malacoplakia, benign prostatic hypertrophy or hyperplasia, and prostate neoplastic disorders, including adenocarcinomas, transitional cell carcinomas, ductal
10 carcinomas, and squamous cell carcinomas.

 Additionally, the compositions of the invention may be useful in the diagnosis, treatment, and/or prevention of disorders or diseases of the penis and urethra, including inflammatory disorders, such as balanoposthitis, balanitis xerotica obliterans, phimosis, paraphimosis, syphilis, herpes simplex virus, gonorrhea, non-
15 gonococcal urethritis, chlamydia, mycoplasma, trichomonas, HIV, AIDS, Reiter's syndrome, condyloma acuminatum, condyloma latum, and pearly penile papules; urethral abnormalities, such as hypospadias, epispadias, and phimosis; premalignant lesions, including Erythroplasia of Queyrat, Bowen's disease, Bowenoid paplosis, giant condyloma of Buscke-Lowenstein, and verrucous carcinoma; penile cancers,
20 including squamous cell carcinomas, carcinoma in situ, verrucous carcinoma, and disseminated penile carcinoma; urethral neoplastic disorders, including penile urethral carcinoma, bulbomembranous urethral carcinoma, and prostatic urethral carcinoma; and erectile disorders, such as priapism, Peyronie's disease, erectile dysfunction, and impotence.

25 Moreover, diseases and/or disorders of the vas deferens include vasculitis and CBAVD (congenital bilateral absence of the vas deferens); additionally, the polynucleotides, polypeptides, and agonists or antagonists of the present invention may be used in the diagnosis, treatment, and/or prevention of diseases and/or disorders of the seminal vesicles, including hydatid disease, congenital chloride
30 diarrhea, and polycystic kidney disease.

 Other disorders and/or diseases of the male reproductive system include, for example, Klinefelter's syndrome, Young's syndrome, premature ejaculation, diabetes

mellitus, cystic fibrosis, Kartagener's syndrome, high fever, multiple sclerosis, and gynecomastia.

Further, the polynucleotides, polypeptides, and agonists or antagonists of the present invention may be used in the diagnosis, treatment, and/or prevention of
5 diseases and/or disorders of the vagina and vulva, including bacterial vaginosis, candida vaginitis, herpes simplex virus, chancroid, granuloma inguinale, lymphogranuloma venereum, scabies, human papillomavirus, vaginal trauma, vulvar trauma, adenositis, chlamydia vaginitis, gonorrhea, trichomonas vaginitis, condyloma acuminatum, syphilis, molluscum contagiosum, atrophic vaginitis, Paget's disease,
10 lichen sclerosus, lichen planus, vulvodynia, toxic shock syndrome, vaginismus, vulvovaginitis, vulvar vestibulitis, and neoplastic disorders, such as squamous cell hyperplasia, clear cell carcinoma, basal cell carcinoma, melanomas, cancer of Bartholin's gland, and vulvar intraepithelial neoplasia.

Disorders and/or diseases of the uterus include dysmenorrhea, retroverted
15 uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhea, Cushing's syndrome, hydatidiform moles, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding (e.g., due to aberrant hormonal signals), and neoplastic disorders, such as adenocarcinomas, leiomyosarcomas, and sarcomas. Additionally, the polypeptides, polynucleotides, or
20 agonists or antagonists of the invention may be useful as a marker or detector of, as well as in the diagnosis, treatment, and/or prevention of congenital uterine abnormalities, such as bicornuate uterus, septate uterus, simple unicornuate uterus, unicornuate uterus with a noncavitary rudimentary horn, unicornuate uterus with a non-communicating cavitary rudimentary horn, unicornuate uterus with a
25 communicating cavitary horn, arcuate uterus, uterine didelphus, and T-shaped uterus.

Ovarian diseases and/or disorders include anovulation, polycystic ovary syndrome (Stein-Leventhal syndrome), ovarian cysts, ovarian hypofunction, ovarian insensitivity to gonadotropins, ovarian overproduction of androgens, right ovarian vein syndrome, amenorrhea, hirsutism, and ovarian cancer (including, but not limited
30 to, primary and secondary cancerous growth, Sertoli-Leydig tumors, endometrioid carcinoma of the ovary, ovarian papillary serous adenocarcinoma, ovarian mucinous adenocarcinoma, and Ovarian Krukenberg tumors).

Cervical diseases and/or disorders include cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, and cervical neoplasms (including, for example, cervical carcinoma, squamous metaplasia, squamous cell carcinoma, adenosquamous cell neoplasia, and columnar cell neoplasia).

Additionally, diseases and/or disorders of the reproductive system include disorders and/or diseases of pregnancy, including miscarriage and stillbirth, such as early abortion, late abortion, spontaneous abortion, induced abortion, therapeutic abortion, threatened abortion, missed abortion, incomplete abortion, complete abortion, habitual abortion, missed abortion, and septic abortion; ectopic pregnancy, anemia, Rh incompatibility, vaginal bleeding during pregnancy, gestational diabetes, intrauterine growth retardation, polyhydramnios, HELLP syndrome, abruptio placentae, placenta previa, hyperemesis, preeclampsia, eclampsia, herpes gestationis, and urticaria of pregnancy. Additionally, the polynucleotides, polypeptides, and agonists or antagonists of the present invention may be used in the diagnosis, treatment, and/or prevention of diseases that can complicate pregnancy, including heart disease, heart failure, rheumatic heart disease, congenital heart disease, mitral valve prolapse, high blood pressure, anemia, kidney disease, infectious disease (e.g., rubella, cytomegalovirus, toxoplasmosis, infectious hepatitis, chlamydia, HIV, AIDS, and genital herpes), diabetes mellitus, Graves' disease, thyroiditis, hypothyroidism, Hashimoto's thyroiditis, chronic active hepatitis, cirrhosis of the liver, primary biliary cirrhosis, asthma, systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, idiopathic thrombocytopenic purpura, appendicitis, ovarian cysts, gallbladder disorders, and obstruction of the intestine.

Complications associated with labor and parturition include premature rupture of the membranes, pre-term labor, post-term pregnancy, postmaturity, labor that progresses too slowly, fetal distress (e.g., abnormal heart rate (fetal or maternal), breathing problems, and abnormal fetal position), shoulder dystocia, prolapsed umbilical cord, amniotic fluid embolism, and aberrant uterine bleeding.

Further, diseases and/or disorders of the postdelivery period, including endometritis, myometritis, parametritis, peritonitis, pelvic thrombophlebitis,

pulmonary embolism, endotoxemia, pyelonephritis, saphenous thrombophlebitis, mastitis, cystitis, postpartum hemorrhage, and inverted uterus.

Other disorders and/or diseases of the female reproductive system that may be diagnosed, treated, and/or prevented by the polynucleotides, polypeptides, and agonists or antagonists of the present invention include, for example, Turner's syndrome, pseudohermaphroditism, premenstrual syndrome, pelvic inflammatory disease, pelvic congestion (vascular engorgement), frigidity, anorgasmia, dyspareunia, ruptured fallopian tube, and Mittelschmerz.

10 Infectious Disease

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial

virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial and fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following Gram-Negative and Gram-positive bacteria, bacterial families, and fungi: Actinomyces (e.g., Norcardia), Acinetobacter, *Cryptococcus neoformans*, Aspergillus, Bacillaceae (e.g., *Bacillus anthraxis*), Bacteroides (e.g., *Bacteroides fragilis*), Blastomycosis, Bordetella, Borrelia (e.g., *Borrelia burgdorferi*), Brucella, Candidia, Campylobacter, Chlamydia, Clostridium (e.g., *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*), Coccidioides, Corynebacterium (e.g., *Corynebacterium diphtheriae*), Cryptococcus, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacter (e.g. *Enterobacter aerogenes*), Enterobacteriaceae (Klebsiella, Salmonella (e.g., *Salmonella typhi*, *Salmonella enteritidis*, *Salmonella typhi*), Serratia, Yersinia, Shigella), Erysipelothrix, Haemophilus (e.g., *Haemophilus influenza* type B), Helicobacter, Legionella (e.g., *Legionella pneumophila*), Leptospira, Listeria (e.g., *Listeria monocytogenes*), Mycoplasma, Mycobacterium (e.g., *Mycobacterium leprae* and *Mycobacterium tuberculosis*), Vibrio (e.g., *Vibrio cholerae*), Neisseriaceae (e.g.,

Neisseria gonorrhea, *Neisseria meningitidis*), Pasteurellaceae, Proteus, Pseudomonas (e.g., *Pseudomonas aeruginosa*), Rickettsiaceae, Spirochetes (e.g., Treponema spp., Leptospira spp., Borrelia spp.), Shigella spp., Staphylococcus (e.g., *Staphylococcus aureus*), Meningiobococcus, Pneumococcus and Streptococcus (e.g., *Streptococcus pneumoniae* and Groups A, B, and C Streptococci), and Ureaplasmas. These bacterial, parasitic, and fungal families can cause diseases or symptoms, including, but not limited to: antibiotic-resistant infections, bacteremia, endocarditis, septicemia, eye infections (e.g., conjunctivitis), uveitis, tuberculosis, gingivitis, bacterial diarrhea, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, dental caries, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, dysentery, paratyphoid fever, food poisoning, Legionella disease, chronic and acute inflammation, erythema, yeast infections, typhoid, pneumonia, gonorrhea, meningitis (e.g., meningitis types A and B), chlamydia, syphilis, diphtheria, leprosy, brucellosis, peptic ulcers, anthrax, spontaneous abortions, birth defects, pneumonia, lung infections, ear infections, deafness, blindness, lethargy, malaise, vomiting, chronic diarrhea, Crohn's disease, colitis, vaginosis, sterility, pelvic inflammatory diseases, candidiasis, paratuberculosis, tuberculosis, lupus, botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections, nosocomial infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, diphtheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Schistosoma, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*). These parasites can cause a variety of diseases or symptoms,

including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be
5 used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an
10 effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997)). The regeneration of
20 tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs
25 (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists
30 of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the

present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular
5 insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases,
10 neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic
15 lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

Gastrointestinal Disorders

20 Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose gastrointestinal disorders, including inflammatory diseases and/or conditions, infections, cancers (e.g., intestinal neoplasms (carcinoid tumor of the small intestine, non-Hodgkin's lymphoma of the small intestine, small bowel lymphoma)), and ulcers, such as peptic
25 ulcers.

Gastrointestinal disorders include dysphagia, odynophagia, inflammation of the esophagus, peptic esophagitis, gastric reflux, submucosal fibrosis and stricturing, Mallory-Weiss lesions, leiomyomas, lipomas, epidermal cancers, adeoncarcinomas, gastric retention disorders, gastroenteritis, gastric atrophy, gastric/stomach cancers,
30 polyps of the stomach, autoimmune disorders such as pernicious anemia, pyloric stenosis, gastritis (bacterial, viral, eosinophilic, stress-induced, chronic erosive, atrophic, plasma cell, and Ménétrier's), and peritoneal diseases (e.g., chyloperitoneum,

hemoperitoneum, mesenteric cyst, mesenteric lymphadenitis, mesenteric vascular occlusion, panniculitis, neoplasms, peritonitis, pneumoperitoneum, bubphrenic abscess,).

Gastrointestinal disorders also include disorders associated with the small intestine, such as malabsorption syndromes, distension, irritable bowel syndrome, sugar intolerance, celiac disease, duodenal ulcers, duodenitis, tropical sprue, Whipple's disease, intestinal lymphangiectasia, Crohn's disease, appendicitis, obstructions of the ileum, Meckel's diverticulum, multiple diverticula, failure of complete rotation of the small and large intestine, lymphoma, and bacterial and parasitic diseases (such as Traveler's diarrhea, typhoid and paratyphoid, cholera, infection by Roundworms (*Ascariasis lumbricoides*), Hookworms (*Ancylostoma duodenale*), Threadworms (*Enterobius vermicularis*), Tapeworms (*Taenia saginata*, *Echinococcus granulosus*, *Diphyllobothrium spp.*, and *T. solium*).

Liver diseases and/or disorders include intrahepatic cholestasis (alagille syndrome, biliary liver cirrhosis), fatty liver (alcoholic fatty liver, reye syndrome), hepatic vein thrombosis, hepatolenticular degeneration, hepatomegaly, hepatopulmonary syndrome, hepatorenal syndrome, portal hypertension (esophageal and gastric varices), liver abscess (amebic liver abscess), liver cirrhosis (alcoholic, biliary and experimental), alcoholic liver diseases (fatty liver, hepatitis, cirrhosis), parasitic (hepatic echinococcosis, fascioliasis, amebic liver abscess), jaundice (hemolytic, hepatocellular, and cholestatic), cholestasis, portal hypertension, liver enlargement, ascites, hepatitis (alcoholic hepatitis, animal hepatitis, chronic hepatitis (autoimmune, hepatitis B, hepatitis C, hepatitis D, drug induced), toxic hepatitis, viral human hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), Wilson's disease, granulomatous hepatitis, secondary biliary cirrhosis, hepatic encephalopathy, portal hypertension, varices, hepatic encephalopathy, primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular adenoma, hemangiomas, bile stones, liver failure (hepatic encephalopathy, acute liver failure), and liver neoplasms (angiomyolipoma, calcified liver metastases, cystic liver metastases, epithelial tumors, fibrolamellar hepatocarcinoma, focal nodular hyperplasia, hepatic adenoma, hepatobiliary cystadenoma, hepatoblastoma, hepatocellular carcinoma, hepatoma, liver cancer, liver hemangioendothelioma, mesenchymal hamartoma, mesenchymal

tumors of liver, nodular regenerative hyperplasia, benign liver tumors (Hepatic cysts [Simple cysts, Polycystic liver disease, Hepatobiliary cystadenoma, Choledochal cyst], Mesenchymal tumors [Mesenchymal hamartoma, Infantile hemangioendothelioma, Hemangioma, Peliosis hepatis, Lipomas, Inflammatory pseudotumor, Miscellaneous], Epithelial tumors [Bile duct epithelium (Bile duct hamartoma, Bile duct adenoma), Hepatocyte (Adenoma, Focal nodular hyperplasia, Nodular regenerative hyperplasia)], malignant liver tumors [hepatocellular, hepatoblastoma, hepatocellular carcinoma, cholangiocellular, cholangiocarcinoma, cystadenocarcinoma, tumors of blood vessels, angiosarcoma, Kaposi's sarcoma, hemangioendothelioma, other tumors, embryonal sarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, carcinosarcoma, teratoma, carcinoid, squamous carcinoma, primary lymphoma]), peliosis hepatis, erythrohepatic porphyria, hepatic porphyria (acute intermittent porphyria, porphyria cutanea tarda), Zellweger syndrome).

15 Pancreatic diseases and/or disorders include acute pancreatitis, chronic pancreatitis (acute necrotizing pancreatitis, alcoholic pancreatitis), neoplasms (adenocarcinoma of the pancreas, cystadenocarcinoma, insulinoma, gastrinoma, and glucagonoma, cystic neoplasms, islet-cell tumors, pancreoblastoma), and other pancreatic diseases (e.g., cystic fibrosis, cyst (pancreatic pseudocyst, pancreatic
20 fistula, insufficiency)).

Gallbladder diseases include gallstones (cholelithiasis and choledocholithiasis), postcholecystectomy syndrome, diverticulosis of the gallbladder, acute cholecystitis, chronic cholecystitis, bile duct tumors, and mucocele.

Diseases and/or disorders of the large intestine include antibiotic-associated
25 colitis, diverticulitis, ulcerative colitis, acquired megacolon, abscesses, fungal and bacterial infections, anorectal disorders (e.g., fissures, hemorrhoids), colonic diseases (colitis, colonic neoplasms [colon cancer, adenomatous colon polyps (e.g., villous adenoma), colon carcinoma, colorectal cancer], colonic diverticulitis, colonic diverticulosis, megacolon [Hirschsprung disease, toxic megacolon]; sigmoid diseases
30 [proctocolitis, sigmoid neoplasms]), constipation, Crohn's disease, diarrhea (infantile diarrhea, dysentery), duodenal diseases (duodenal neoplasms, duodenal obstruction, duodenal ulcer, duodenitis), enteritis (enterocolitis), HIV enteropathy, ileal diseases

(ileal neoplasms, ileitis), immunoproliferative small intestinal disease, inflammatory bowel disease (ulcerative colitis, Crohn's disease), intestinal atresia, parasitic diseases (anisakiasis, balantidiasis, blastocystis infections, cryptosporidiosis, dientamoebiasis, amebic dysentery, giardiasis), intestinal fistula (rectal fistula), intestinal neoplasms (cecal neoplasms, colonic neoplasms, duodenal neoplasms, ileal neoplasms, intestinal polyps, jejunal neoplasms, rectal neoplasms), intestinal obstruction (afferent loop syndrome, duodenal obstruction, impacted feces, intestinal pseudo-obstruction [cecal volvulus], intussusception), intestinal perforation, intestinal polyps (colonic polyps, gardner syndrome, peutz-jeghers syndrome), jejunal diseases (jejunal neoplasms), malabsorption syndromes (blind loop syndrome, celiac disease, lactose intolerance, short bowl syndrome, tropical sprue, whipple's disease), mesenteric vascular occlusion, pneumatosis cystoides intestinalis, protein-losing enteropathies (intestinal lymphagiectasis), rectal diseases (anus diseases, fecal incontinence, hemorrhoids, proctitis, rectal fistula, rectal prolapse, rectocele), peptic ulcer (duodenal ulcer, peptic esophagitis, hemorrhage, perforation, stomach ulcer, Zollinger-Ellison syndrome), postgastrectomy syndromes (dumping syndrome), stomach diseases (e.g., achlorhydria, duodenogastric reflux (bile reflux), gastric antral vascular ectasia, gastric fistula, gastric outlet obstruction, gastritis (atrophic or hypertrophic), gastroparesis, stomach dilatation, stomach diverticulum, stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, hyperplastic gastric polyp), stomach rupture, stomach ulcer, stomach volvulus), tuberculosis, visceroptosis, vomiting (e.g., hematemesis, hyperemesis gravidarum, postoperative nausea and vomiting) and hemorrhagic colitis.

Further diseases and/or disorders of the gastrointestinal system include biliary tract diseases, such as, gastroschisis, fistula (e.g., biliary fistula, esophageal fistula, gastric fistula, intestinal fistula, pancreatic fistula), neoplasms (e.g., biliary tract neoplasms, esophageal neoplasms, such as adenocarcinoma of the esophagus, esophageal squamous cell carcinoma, gastrointestinal neoplasms, pancreatic neoplasms, such as adenocarcinoma of the pancreas, mucinous cystic neoplasm of the pancreas, pancreatic cystic neoplasms, pancreatoblastoma, and peritoneal neoplasms), esophageal disease (e.g., bullous diseases, candidiasis, glycogenic acanthosis, ulceration, barrett esophagus varices, atresia, cyst, diverticulum (e.g., Zenker's

diverticulum), fistula (e.g., tracheoesophageal fistula), motility disorders (e.g., CREST syndrome, deglutition disorders, achalasia, spasm, gastroesophageal reflux), neoplasms, perforation (e.g., Boerhaave syndrome, Mallory-Weiss syndrome), stenosis, esophagitis, diaphragmatic hernia (e.g., hiatal hernia); gastrointestinal
5 diseases, such as, gastroenteritis (e.g., cholera morbus, norwalk virus infection), hemorrhage (e.g., hematemesis, melena, peptic ulcer hemorrhage), stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, stomach cancer)), hernia (e.g., congenital diaphragmatic hernia, femoral hernia, inguinal hernia, obturator hernia, umbilical hernia, ventral hernia), and intestinal diseases (e.g., cecal
10 diseases (appendicitis, cecal neoplasms)).

Chemotaxis

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or
15 mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotaxic activity of particular cells. These
20 chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by
25 attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or
30 polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit
5 (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural
10 or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

15 Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or
20 inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

25 Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a
30 standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The

antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labeled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-

33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998); each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and
5 corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior
10 to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments,
15 the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation
20 factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present
25 invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the
30 polypeptide of the present invention, the compound to be screened and $^3\text{[H]}$ thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be

screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of $^3\text{[H]}$ thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of $^3\text{[H]}$ thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

5 As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention
10 (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate
15 episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

20 By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes
25 known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a
30 non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of

benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

5 **Drug Screening**

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected
10 compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in
15 such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation
20 of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the
25 present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a
30 particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present

invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

10 This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Polypeptides of the Invention Binding Peptides and Other Molecules

20 The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind polypeptides of the invention, and the polypeptide of the invention binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the polypeptides of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

25 This method comprises the steps of: contacting a polypeptide of the invention with a plurality of molecules; and identifying a molecule that binds the polypeptide of the invention.

30 The step of contacting the polypeptide of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the polypeptide of the invention on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized polypeptide of the invention. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized polypeptide of the

invention. The molecules having a selective affinity for the polypeptide of the invention can then be purified by affinity selection. The nature of the solid support, process for attachment of the polypeptide of the invention to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional
5 and well known to those of ordinary skill in the art.

Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of
10 polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the polypeptide of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the polypeptide of
15 the invention and the individual clone. Prior to contacting the polypeptide of the invention with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of
20 transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for a polypeptide of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the polypeptide of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be
25 determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound
30 polypeptide of the invention, or alternatively, unbound polypeptides, from a mixture of the polypeptide of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction.

Such a wash step may be particularly desirable when the polypeptide of the invention or the plurality of polypeptides is bound to a solid support.

The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind to a polypeptide of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., 1991, *Science* 251:767-773; Houghten et al., 1991, *Nature* 354:84-86; Lam et al., 1991, *Nature* 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-710; Gallop et al., 1994, *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., 1992, *Biotechniques* 13:412; Jayawickreme et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

Examples of phage display libraries are described in Scott and Smith, 1990, *Science* 249:386-390; Devlin et al., 1990, *Science*, 249:404-406; Christian, R. B., et al., 1992, *J. Mol. Biol.* 227:711-718; Lenstra, 1992, *J. Immunol. Meth.* 152:149-157; Kay et al., 1993, *Gene* 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, *Proc. Natl. Acad. Sci. USA* 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is

great. For example, Ecker and Crooke, 1995, *Bio/Technology* 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of
5 various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the
10 scaffold might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side
15 chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

20 Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, *Adv. Exp. Med. Biol.* 251:215-218; Scott and Smith, 1990, *Science* 249:386-390; Fowlkes et al., 1992; *BioTechniques* 13:422-427; Oldenburg et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu et al.,
25 1994, *Cell* 76:933-945; Staudt et al., 1988, *Science* 241:577-580; Bock et al., 1992, *Nature* 355:564-566; Tuerk et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington et al., 1992, *Nature* 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, *Science* 263:671-673; and CT Publication No. WO 94/18318.

30 In a specific embodiment, screening to identify a molecule that binds a polypeptide of the invention can be carried out by contacting the library members with a polypeptide of the invention immobilized on a solid phase and harvesting those

library members that bind to the polypeptide of the invention. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

5 In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to a polypeptide of the invention.

10 Where the polypeptide of the invention binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

15 Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine.

20 Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

25 As mentioned above, in the case of a polypeptide of the invention binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a polypeptide of the invention binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

30 The selected polypeptide of the invention binding polypeptide can be obtained by chemical synthesis or recombinant expression.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited
5 clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA
10 or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300
15 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These
20 experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complementary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is
25 heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl₂, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA
30 oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense

RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or
5 a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard
10 in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early
15 promoter region (Bernoist and Chambon, Nature, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A., 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., Nature, 296:39-42 (1982)), etc.

20 The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA,
25 forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the
30 invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at
5 inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non-translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should
10 include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50
15 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or
20 phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.*, 84:648-652 (1987); PCT Publication
25 NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, *e.g.*, Krol et al., *BioTechniques*, 6:958-976 (1988)) or intercalating agents. (See, *e.g.*, Zon, *Pharm. Res.*, 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide,
30 hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are

commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5' -UG-3' . The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of

the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

5 Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

10 The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

15 The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

20 Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

25 invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention

Other Activities

30 The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide

may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to

modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by
5 influencing biorhythms, circadian rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present
10 invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Other Preferred Embodiments

15 Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous
20 nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous
25 nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of
30 contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5'

Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide
5 sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

10 A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X
15 in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under
20 stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which
25 comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide
30 sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA

Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected

from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said
5 cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit
10 with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the
15 deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA
20 clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as
25 defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a
30 polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as

defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid

sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone

identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred
5 is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is
10 expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of
15 the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA
20 clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to
25 increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit,
30 goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 7, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 7 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 7. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 7. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 7

Gene No.	cDNA Clone ID	NT SEQ ID NO: X	Contig ID	Public Accession Numbers
1	HFKLX38	11	1132442	AI245696, AI250552, AA410788, AI223626, AI251034, AI284543, AA847499, AW303098, AA644090, AI061313, AI254779, AW341978, AW023111, AI654738, AI923050, AI284640, AA602906, AA833875, AA833896, AI801505, AA515728, AA704393, AA643770, AI249853, AL038936, AL040054, AI254770, AI755202, AI066646, AA721645, AI745151, AI962030, AA904275, AA574442, AI244127, AL037910, AI345681, AI345675, AA053463, AA226584, AW088462, AA610509, AW276678, AI499954, AA503298, AI040051, AI754170, AA536040, AI926728, AW117740, AI792529, AI885572, AI278440, AA828867, AI277373, AI932443, AI054193, AI267356, AI267450, AA228778, AI753113, AA315361, AI334896, AW328446, AA714011, AI675615, AA630854, AW069412, AA622801, AI056177, AI133083, AP000694, AL035587, AC005939, Z85987, AC005736, AC004643, Z83844, AC003010, AC003688, AC005932, AL031622, AC006014, AC005921, AP000695, AL117344, AC004257, AC005488, U73640, AC012384, AC005670, AC006441, AC005846, Z97054, AC007774, AC006236, AC003084, AP000504, AC007566, AL035683, Z99716, AL021579, AL121694, AL031295, AC002985, AC002470, AF031078, AC005375, AC005520, AC005539, AC004895, AC005274, AC002316, AF196972, AC005225, AC002132, AL022476, AF030876, AC000134, AC004647,

				AC002472, AC005566, AC004797, AP000885, U47924, AC007664, AL133245, AL008729, AC005369, AC004914, U82828, AL079340, AC006576, AL031432, AF001550, AL050307, AL022320, AC000364, U67817, AC006449, AC004263, AC005914, Z83847, AC005907, AC005666, AC002126, AP000513, AC002468, AC005057, AL109952, Z82195, AL033527, AL034420, Z70280, AC007999, AC004966, AC006211, L44140, AF047825, AC005183, AL035455, AL109827, AF129756, Z97352, AL022098, AC002404, AC002504, AL049839, AC005702, AL133445, AC004859, AC004805, AC004799, AP000495, AC004526, AC004492, AJ251973, Z93848, AC005200, AL034429, AL031283, AL031584, AC007201, Z82217, AD000813, AC002476, AL121825, AC007298, AC005399, AL133246, AL096701, AC005318, AL031670, AC007462, AC004400, Z98753, AC004505, AL020989, AC004955, AC005619, AC005952, AC006965, AC005800, AC005899, AC002116, AL049872, AB023053, AC004150, AL049589, AC003967, AC002054, AL023553, AC002554, AC005484, AC004841, AD000812, AF064858, Z94056, AC000031, AC004144, AL023883, AC005971, AF205588, Z95115, AL136295, AC007011, AC009247, AC006241, AL096791, Z99128, U96629, AL021154, AL023577, Z83845, Z99127, AP000516, AC007536, AP000503, AC006530, AC005740, AC007845, Z97056, AC004020, AC005102, AC006138, AP001050, AC006088, AL050312, AL008730, L47234, AL035420, AC008018, U07561, AL022315, AC004024, AL031431, AL009181, AC009248, AC006509, AC004031, AC007993, Z83851, AC007388, AP001052, AL049776, AC005694, AC004832, AL031846, AL049694, AC005796, AC007065, AP000014, AC005786, U63721, AC005874, AF134471, AC007277, AC007242, AL022316, AC008041, and AC003046.
1	HFKLX38	33	1042908	AI245696, AA847499, AA410788, AI250552, AI223626, AW023111, AI251034, AI284543, AI254779, AI061313, AW303098, AA644090, AI284640, AI654738, AI923050, AA602906, AW341978, AI801505, AA503298, AA833875, AA833896, AA643770, AA704393, AA515728, AL038936, AI254770, AL040054, AW069412, AI056177, AI249853, AI755202, AI066646, AI745151, AA622801, AI962030, AA904275, AW419389, AA721645, AA630854, AI345681, AI345675, AA574442, AI244127, AL037910, AI792529, AI278440, AI277373, AW088462, AI334896, AI040051, AI583466, AI926728, AI332615, AI054193, AA610509, AW276678, AW328446, AA053463, AA226584, AI267356, AI267450, AI499954, AA536040, AI754170,

				AI932443, AI753113, AI675615, AW117740, AI885572, AA828867, AI435639, AA228778, AA714011, AA315361, AL035587, AC005939, Z85987, Z83844, AC003010, AC005736, AC005932, AC006014, AC005921, AL031622, AC004257, AL121653, AC005488, AL035683, AC005670, Z97054, AL117344, AC005539, AL133245, U73640, AF031078, AC012384, Z99716, AC007774, AC007664, AC003084, AC002132, AC004895, AC002985, AF030876, AC006441, AC005846, AL021579, AL121694, AC006236, U47924, AC002472, AP000504, AC002470, AC004914, AC005520, AC005274, AC002504, AC005225, AC000134, AL133445, AC007566, AC006576, U82828, AL022476, AC005666, AC005057, AC002126, AL033527, AL008729, AL109952, AL031432, Z97352, AC002316, AL022320, AC005702, AC004263, AC007999, AC004647, AC005619, AC005566, AC006241, AC005914, AC004797, U67817, Z83847, AC005907, AP000885, AP000513, AL031295, AL034420, U78027, Z82217, Z70280, AC007201, AC004020, AL031431, AC006211, AC004966, AC007845, L44140, AC007298, AF047825, AF001550, AL050307, AL109827, AF129756, AL035455, AC004832, AL022098, AC005632, AC000364, AC006449, AC016025, AC004874, AL049589, AC005237, AC005484, AC005200, AP000495, AC007011, Z94056, AC004526, AC004805, AC002468, AP000503, AL109628, AC004031, AL031584, AJ251973, AP000133, AP000211, AC006530, Z93848, AL031283, AL034429, AC005696, AL049646, AC005740, AC007388, AL035422, AD000813, AC007193, AC005183, AL023553, AC006509, AC004841, AC007227, AL121825, AL133246, AC005399, AL096701, AL009181, AL031657, AC002404, Z98753, AC004505, AL020989, AL078477, X54156, U94788, AC007993, AC004955, AC002044, M89651, AC005899, AL022316, AC004144, AL132777, AL049872, AC002116, AC004150, AB023053, AC009247, AC005800, AC006088, AC003991, AL079340, AC007690, AC002054, AC002554, AC003967, AC004883, AL035420, AD000812, AC004492, AC000031, AL023883, U07561, AC005952, AF205588, AC005971, Z97196, AL135744, AL136295, Z99127, AL008627, AL096791, AL049839, AC005786, Z98946, AC007065, AC005082, AC005796, AC007537, AL121603, AF134726, AC004400, AL023577, AC005300, AP000516, U62293, AC007536, AC005011, AL008582, AC005050, AC002476, AC004799, Z82195, AC005519, AL035681, and AJ246003.
2	HUVFH14	12	1235257	AA442703, AW274009, AI631143, AW193700, AI765240, AI291446, AI567672,

				AI351633, AI910455, AW023476, AA452266, AA451911, N30578, AA452435, AI376609, H93102, AA496039, AI291783, AA652658, AW029172, AA478485, AL036477, AW183375, AA861912, AA746596, R77622, AI308068, N57324, H95123, H93230, N32228, H61522, N42045, AA452091, N32904, AI015768, H94000, R68550, AW243220, AL121308, H72146, AA804847, AI475438, R56241, AI273909, R68517, Z43920, H72057, AA187820, R74274, R77623, AA369265, Z39976, R68221, R68256, R74187, T35057, AA247560, D12234, Z21582, F13647, AI557751, D59889, C14429, C14227, D80949, D81111, D80196, D80188, D80212, D58283, D80391, D59275, T03269, D80166, D59859, D80195, D81030, D59927, D51423, D59619, D80219, D80210, D51799, D80168, D80240, D80253, D80269, D80227, D80193, D80043, D80045, D80038, D80366, D57483, D80022, C14331, D50995, D50979, C14407, D80378, D80024, D59502, D59787, C14014, C75259, D59610, D80241, C15076, D80164, D80134, C14389, D80064, C14298, AW089844, D59467, D80268, N71180, D52291, AI690813, C06015, AA305409, T02974, AA676361, AI560954, D51213, AI312040, N81164, D81026, AI453767, D80014, AI804505, D51022, AI473799, AI582912, AA427700, AL045986, AI927233, AI370623, AI440238, AA764900, AI621341, AW078606, AI539690, AW050850, AW194014, AW075382, AI830016, AW198090, AL079799, AI570140, T11417, AW083572, AI571439, AI802542, AI633125, AI479292, AA761557, AI254814, H89138, AI921746, AI473536, AI151101, AI538850, AI866090, AW085393, AL047100, AW058202, AI929108, AI537187, AI923750, AW020397, AA305578, AI804531, AA514684, AI434731, AI583578, AW051088, AL039274, AL046942, AA437338, AW161098, AI559752, AI687482, AL046466, AI679550, AI270183, AI590043, AW131228, AW019985, AI699020, AL037602, AI114703, C14077, AI799313, AI687809, AA829930, AI860027, AI887268, AI352514, AA825548, AI623941, AI608743, AI559619, AI783825, AI890907, AA809122, AW166870, AI401697, AL037582, AW080742, AI698491, AI580694, AI648458, AI673363, AI345415, AW103923, AW103200, AI565889, AW084046, AI683348, AI274614, AI917428, AW163554, AL033377, AL080079, D17111, A62298, A84916, A62300, X68127, Z82022, AJ132110, AR018138, X67155, A67220, D89785, A78862, I82448, A25909, A70386, D88547, AR016808, AR025207, AL133049, X64588, AR013797, AL137533, AL133015,
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2	HUVFH14	35	1042382	<p>AA442703, AW274009, AI631143, AW193700, AI765240, AI291446, AI567672, AI351633, AI910455, AW023476, AA452266, AA451911, N30578, AA452435, AI376609, H93102, AA496039, AI291783, AA652658, AW029172, AA478485, AL036477, AW183375, AA861912, AA746596, R77622, AI308068, N57324, N32228, H95123, H93230, N42045, H61522, AA452091, N32904, AI015768, H94000, R68517, R68550,</p>

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3	HWMMAH36	13	1143882	AI676060, AA527544, AI671947, AW375902,

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3	HWMHAH36	36	1033673	AW375902, AA527544, AW375746, and AF071777.
4	HUVHI35	14	1145693	AA843298, AI459124, AW190431, AI660576, AA007470, AW410587, AW374284, N27976, AI220560, F30587, AA527440, AI707992, AI239425, AA011457, AW183785, AW102678, AA936247, AI027344, AA033809, AA303710, AA506343, W42889, W19156, N99944, AA741435, AW135271, AA972615, F36520, AA007308, AW304475, W42774, AI298256, N21322, W31096, AI469654, AA994238, AA011400, AA425283, AI439525, AI224583, AW021674, AW151541, AA180056, AI798521, AI791659, AW020150, AI521525, AI254267, AI251024, AA526542, AI590404, AI570067, T03928, AI860423, AW243817, AW419201, AA601376, AI049999, AW275432, AI801563, AA533660, AW409621, AW403177, AA489390, AA610644, AI590442, AI884404, AI926102, AA568303, AW023390, AI065031, AA584360, AI355246, AA630476, AI620666, AA595661, AA828840, AI924950, AW272815, AI819419, AA133568, AL138455, AI252005, AI254463, AA569220, AA218684, AA493546, AA534193, AI754926, AI434103, AA720582, AI828721, AI061313, AW072963, AI567676, AA676592, AL039436, AA632355, AI890297, AI797998, AW338376, AA171400, AI114657, AI123488, AW439224, AI114494, AW328331, AW409626, AI753131, AA640305, AA831714, AW025064, C14614, N26159, AI285651, AW182182, AI307563, AA313025, AA132929, AI761677, AI251696, F23338, AA167656, H62123, AA603359, AI572680, AA935827, AA199578, AI860648, H47461, AI745666, AI270280, AI708108, AI869797, AL044458, AL042731, AW020094, AA507623, AA760655, AI078409, AW238341, AW338633, AW069769, AA525753, AW021399, AA084320, AI332615, AA829565, AA558488, AW302048, AI031759, AI609992, AC005594, AC005874, AF134471, AC005696, AL132987, AC005911, AC006211, AL022322, AL009183, AC004841, AL035422, U78027, Z98200, AC004477, AC002115, AC004659, U91322, Z84469, Z83844, AC010077, AL031311, AC005291, AC007066, AC004134, Z93023, Z82190, AL031281, AC009731, AC006006, AC005288, Z98744, AL034402, AC004929, U91326, AL008582, AF064863, AC004883, AC003030, AP000338, AL035398, AC002059, AC006121, AP000216, AE000658, D84394,

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4	HUVHI35	37	1145508	<p>AI459124, AA843298, AW190431, AI660576, N27976, AW410587, AA007308, AI298256, AI220560, AA007470, AW374284, AA527440, F30587, AI707992, AI239425, AA936247, AW102678, AA011457, AW183785, N21322,</p>

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4	HUVHI35	38	1034948	AW374284, AA534193, AW410587, AI830367, AI360265, AC005594, and AC005783.
4	HUVHI35	39	1042477	AI459124, AI660576, AW190431, AA843298, AA007308, AA007470, AI298256, AA936247, N21322, AI220560, AA527440, W19156, AI707992, AI239425, AA011457, AW102678, F30587, AW183785, AI027344, N99944, AA506343, AA741435, AW135271, W42774, AA708616, AA303710, AA033809, AA989079, AA011400, W42889, AA972615, AI469654, F36520, AW304475, N27976, AA994238, AA527413, AA876698, AW410588, W31096, AI446371, AA928285, AA659692, AA507946, AA016145, AA910748, AA625607, AI927725,

				W25280, AA877297, AC005594, and AA994683.
5	HHFML08	15	1108125	AI420422, AA622711, AA635809, and AI167807.
5	HHFML08	40	1105717	AI420422, AA622711, AA635809, and AI167807.
5	HHFML08	41	1042414	AA622711, AA635809, AI420422, and AI167807.
6	HE2KK74	16	1131126	AI026821, AA503776, AI435527, AW298357, AI767392, AI142574, AI094514, AW073866, AL036946, AA206595, AA969442, AA040034, AW241144, AA814156, AA354909, AA933895, AA040828, D51799, D80227, C01416, D80212, D80188, D58283, D59889, C14429, D80166, D51423, D59619, D80210, D80240, D80253, D80043, D80195, D80219, D81030, D80391, D59859, D80269, D50979, D80196, D59610, D59927, D80038, D80193, D80366, D80241, D59275, D80045, D80022, D57483, C75259, C14014, C14331, D80378, D59502, D50995, T03269, D80024, D80164, D59787, D59467, D80134, D51060, C14389, D80268, F13647, AA305409, C15076, D51250, D58253, D81111, D81026, AW178893, D80949, D80168, D51079, C14227, AW177440, D51022, AW179328, AI557751, AA305578, AW178775, AW378532, D80522, AI910186, C14407, AW377671, D80248, AW352158, Z21582, D80251, D59695, AA285331, AI905856, AW369651, D51097, D52291, AW177501, AA514188, AW178762, AW177511, AA514186, C14298, D80133, D80064, AW360811, AW360834, C05695, AW352117, AW176467, AW375405, AW378540, AW360844, AW366296, AW360817, AW375406, AW378534, AW179332, AW377672, AW179023, AW178905, D80132, D80439, D59373, D80014, T11417, D80302, AW352171, AW377676, AW178906, AW352170, AW177731, AW178907, AW179019, AW179024, D80247, AW177505, AW360841, AW179020, AW178909, AW177456, AW378528, AW179329, AW178980, AW177733, AW178908, AW178754, AW179018, AA809122, D51103, AW352174, D51759, AW179004, T02974, D80157, AW179012, AW178914, AW378525, AW177722, AW378543, AW177728, D51213, T03116, AW179009, AW367967, AW178774, AW178911, AW352163, C06015, D58246, AW178983, AW352120, AW378539, AW178781, T48593, D58101, D59653, D80258, D59503, AI557774, AW177723, D45260, C14344, AI535850, D59627, H67854, AW177508, C14975, C03092, AW367950, AI535686, H67866, AW378533, AI525923,

				AA033512, D59317, AW178986, AW177497, AI525917, AA514184, D59474, AI525920, AW179220, D51221, AW177734, AI535961, D80228, C14957, C14973, AI525928, AI525235, D60010, N66429, D45273, D59551, D50981, AI525227, AI525912, A62298, A84916, A62300, AJ132110, Y17188, X67155, AR018138, A67220, A25909, D89785, A78862, D26022, D34614, D88547, AR025207, X82626, AR008278, X68127, AF058696, AB028859, X93549, AB012117, A82595, A85396, Y12724, AR066482, A85477, AR016808, A44171, A86792, A30438, I19525, U87250, AF135125, A94995, AR060385, AB002449, AR008443, I50133, Y17187, I50126, I50132, I50128, AR066488, AR016514, AR060138, A45456, A26615, AR052274, AR008277, AR008281, AR064240, Y09669, A43192, A43190, AR038669, AR066487, U46128, AR016691, AR016690, AR066490, U79457, D88507, I14842, AR054175, I18367, D50010, AB033111, A63261, AR008408, AR062872, A70867, D13509, A64136, A68321, AR060133, Z32749, I79511, U87247, AB023656, AF123263, AR032065, X93535, and AR008382.
6	HE2KK74	42	1127450	AI026821, AA503776, AI435527, AW298357, AI767392, AI142574, AI094514, AW073866, AL036946, AA206595, AA969442, AA040034, AW241144, AA354909, AA814156, AA933895, AA040828, and C01416.
6	HE2KK74	43	1043915	C01416, AA206595, and AA933895.
7	HFKME15	17	1094386	T93560, AA062982, AI354847, AA634991, AW406447, AL048969, AW157180, AI929298, AI929825, AI859742, AI889426, AI275631, AA584482, AA742815, AI433104, W04193, AA130647, F30357, AI860423, AA569182, AA020873, AA502511, AI583978, AI457152, AA836811, AA457754, AA595206, AI797998, AA192330, AA614180, AI310343, AA228349, AI801563, H18825, AI499134, AA470512, AW275432, AI355246, AI745666, AI433952, AA666048, AI174703, AA630476, AA236867, AA568314, AW272815, AI572680, AA574286, AI360521, AA811451, AW419389, AI123488, AA525807, AI761677, AW021674, AC006312, AC003043, AC006273, AC007055, AC006011, AC005899, AL133448, AC002316, AC004821, AL024498, AC006211, AC006501, AL035587, AC005081, AC003982, AC009516, Z86090, AC005527, AF165926, AL022323, AP000553, AC005913, U95742, AL080243, AC005726, AL034420, AL023575, AC006084, AL035659, AC005088, AC005071, AB020868, AP000311, AF129756, AC005971, AL031005, AL023807, AC004000, Z85987, AC005225, Z95331, AC005839, AC007298, AC004799, AL049869,

				AC000025, AC007216, AC000353, AC005529, AC006251, AC008040, AC004099, AC005746, AC007563, AC004820, AC007151, AJ003147, AC005003, AP000065, AL008726, AC005288, AP000512, AC007193, AC006449, Z84480, AC005800, AC007384, AL031662, AL139054, AF001549, AC005300, U95739, AC003029, AL049766, AL121658, AC002310, AC005089, AL121754, AP000049, AL022326, AL031289, AC006480, AP000555, Y10196, AL031053, AF053356, AC004678, AL031680, AP000694, AC002400, AP000558, AL049843, AC005180, AC005255, AL022334, AC016027, AP000692, AL009181, AL031848, AC006581, AC007041, AC002430, AC005067, AL121652, AC005914, AC002470, AL121603, AL031228, AL022165, AC016025, AL034351, AC005722, AF038458, AL049780, AC007421, AC007386, AC005291, AC006285, AC005516, AF196779, AC002301, AC005484, AP000114, AC005520, Z98884, AL049759, AL133500, AC002039, Z98200, AL109628, AB000882, AC005694, AL031281, AC012384, AC004526, AC009498, AL031311, AC005755, U85195, AL031133, U91326, AC002544, AC008101, AC004659, AL035443, AC002073, AC006468, AL078476, AC002312, AC005082, AL132712, AC002306, AF045555, AC004967, AC004686, AL049589, AL020997, AC003101, AL021546, AC005228, Z84469, AF134726, AC004883, AC002059, AC005632, Y18000, AC005911, AP000359, AC005512, AE000658, AC007546, AP000501, AC005740, U52112, AC005057, AL022311, AL031283, AC006538, AC005280, AL035495, AC003041, AC016830, Z82215, AC006057, AC005215, U47924, AP000346, AF205588, AL133353, AC007565, AC004382, AC002468, AC005060, AL109827, AC002302, AC005486, AC008079, AC005940, AF196971, AL022320, AB023051, AC004854, AL034451, AP000689, AC005377, AL049795, AC003102, AC005531, U62293, AL021154, AC003665, Z82180, AL031577, AL080241, AL034417, U63721, AL096791, AC003007, AF196972, AC004253, AL035684, AC006121, Z99716, AB003151, Z93017, AF207550, AC005921, AL109656, AP000509, AL034421, AC005332, AC004531, AC006137, AC004216, AC004771, AL109627, AC003688, AC006530, AC007308, AF109907, AC004797, AL079295, AC005972, AC002072, Z93241, AL133244, AC005399, AC007279, AC006213, and AC004673.
7	HFKME15	44	1034681	T93560, AA062618, AA062982, AA287618, AA836811, AW406447, F24792, AA748048, AI433104, AA773302, AI061111, AA192330, AC006011, AC007934, Z85987, AC006312, AC006273, AC005255, AC005899, AL133500,

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8	HE2LW65	18	1222608	AA447258, AA449169, AI935666, AL134430, AA044672, AI310530, AA633864, AI608963, AA648999, AI828216, AI283033, AI638071, AA770541, AI079280, AI469994, AI214200, AI073527, AA617801, AA083480, AI752992, AI366846, AA403045, AL134429, AI278917, AI214262, AA476497, AA044748, AW082904, AI310353, AW102586, N62870, W56187, AA922307, AW242267, AW387708, T65498, AA505952, AW190878, T74360, AI888954, AA083572, H26998, H27999, N77776, AA315729, H45347, AI359218, AA863144, D81939, T33354, W56264, AA482897, H45277, R64447, AI283900, H30190, T74969, AW167352, AA634813, H08801, AA379961, AA350289, Z44835, F12824, F11825, F10427, F12680, AA747725, AI364881, F10291, H97948, AA296743, AA379902, T65421, T17138, AA045015, AA521158, R43571, AA371289, AA961616, AA452536, AA382990, R63835, N72159, Z40618, AA382991, AW387707, AI702525, AA447134, AI984512, AA648967, AA648951, AW001342, AI264784, AA905187, AA657615, AI304637, Z45627, AA045014, AW375723, AA368261, AA679694, AI431870, AA523370, AA548928, AA994756, AA594393, AC002486, AC004837, AF086082, L19698, X04328, X15014, M29893, Z48587, AC004805, AP000042, AP000110, AC005304, AP000289, and AC002044.
8	HE2LW65	45	1035079	AC004837
8	HE2LW65	46	1042434	AA447258, AL134430, AI935666, AA044672, AI310530, AA633864, AI608963, AI828216, AI283033, AI638071, AA770541, AI079280, AI469994, AI214200, AI073527, AA617801, D81939, AI752992, AA083480, AI366846, AI278917, AI214262, AA476497, AA044748, AW082904, AW102586, AI310353, W56187, AA922307, AW242267, AW387708, AW190878, AA505952, AI888954, AA083572, H27999, H26998, H45347, AA315729, AA863144, AI359218, W56264, T33354, AA482897, H45277, R64447, AI283900, H30190, AW167352, AA634813, H08801, AA379961, F10427, AA747725, AI364881, H97948, F10291, AA296743, AA379902, AA045015, T17138, AA521158, R43571, AA371289, AA961616, R63835, AA452536, N72159, Z40618, AA449169, AW387707, AI702525, AA447134, T65421, AA648999, AA382990, AA382991, AA657615, AI304637, AA045014, AW375723, H94944, AA368261, C15495, AA679694, AA188426, AI431870, AC004837, AC002486, AL080170, AC004805, AC002044, AP000289, AW468167, AW474027, AW513758, AW515483, AW515487, and AW594459.

9	HTPHS66	19	1179778	AW189885, AW384901, AW340739, AI972342, AI358874, AI243359, AA828004, AW079912, AI088690, AI040077, AA532364, W76069, AA604083, W72511, AI347052, AW384886, AA678070, AA292131, AI750441, AI129871, AI245928, AA292132, AA704401, AI750440, H88655, D81553, AW244162, H88587, AA375083, H88539, AA328473, AW062607, H88270, AA961517, AA320829, R68257, R68551, AA584313, H88699, AA329081, N63129, H88271, AA342606, F13763, AW078502, AI424628, C01861, AI369507, D80045, C14331, D51799, D80253, C14429, D80366, D80391, D59787, D59859, D58283, D81030, D80166, D57483, D80227, D51423, D59619, D80210, D80240, D80196, D80188, D59467, D80212, D59610, D80022, D80043, D80195, D59889, D80219, D80269, D80164, D59275, D59502, D59927, D50995, D80378, D80038, D80241, D80024, C14389, D50979, D80193, AI905856, C75259, C15076, AA305409, AW366296, D51060, T03269, AW178893, C14014, AI557751, D51022, AW179328, AW178775, D80134, D80251, AW375405, AW378532, D51250, AW177440, D80522, AA305578, C14407, D81026, D58253, D80268, AW352158, D80133, F13647, D80248, AA121634, AW369651, D80949, C14227, AW178762, AA514188, Z21582, D80168, D51079, D81111, AA514186, AI910186, AW177501, AW177511, AW375406, AW360811, C05695, C14298, D80064, AW352117, AW176467, AI535660, AW378540, AW377671, AW360844, AW360817, AW378534, AW179332, AW377672, AW179023, AW178905, D51097, D80439, D80132, AW360834, D59373, AI557241, D80302, T48593, AA285331, AW352171, AI535686, AW377676, D80247, AW178906, AW352170, AW177731, AW178907, AA809122, AW179019, AW179024, AW177505, AW360841, AW179020, AW178909, AW177456, AW178980, AW179329, AW177733, AW378528, AW178908, AW178754, AW179018, AI536138, AI535639, AW179220, AW179004, D80157, AW179012, AW178914, AW352174, AW378525, D51103, T11417, D51759, AI525856, AW177722, AW177728, AW179009, AW178774, AW178911, AW378543, AW352163, AI557238, AW367967, D80014, C06015, AI557258, AW178983, AI557808, AW352120, D58246, AW178781, D58101, AI541027, D59653, AW177723, D45260, D59503, AI557731, C14344, T03116, AI557774, AI541205, D80258, AI541048, D51002, D59627, H67854,
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				AL137471, A62298, A62300, AR018138, A84916, Y17188, A82595, AJ132110, X67155, D26022, A25909, A67220, D89785, X82626, A78862, D34614, Y17187, D88547, AF058696, U87250, AR008278, A30438, AB028859, A94995, AR025207, X68127, Y11505, Y12724, S68736, AB012117, A44171, AR016514, A43190, A85396, AR066482, U79457, A85477, AR060385, I19525, A86792, Y08991, AB002449, AR008443, X93549, I50126, I50132, I50128, I50133, AR038669, AF135125, AR025466, AR008277, AR008281, AR066488, AR060138, A45456, A26615, AR052274, Y09669, A43192, AR066487, AR066490, D88507, I14842, AR054175, U46128, D50010, AR050070, I18367, AR016691, AR016690, A63261, A91160, AR008408, AR062872, A70867, AB033111, D13509, A82593, A64136, A68321, AR060133, AR064240, I79511, Z30183, U94592, U87247, U45328, AB023656, and AF123263.
9	HTPHS66	47	1151444	AW189885, AW384901, AW340739, AI972342, AI358874, AA828004, AI243359, AW079912, AI088690, AI040077, AA532364, W76069, AA604083, W72511, AI347052, AW384886, AA678070, AA292131, AI750441, AI129871, AI245928, AA292132, AA704401, AI750440, H88655, D81553, AW244162, H88587, AA375083, H88539, AA328473, AW062607, H88270, AA584313, AA961517, AA320829, R68257, R68551, H88699, AA329081, N63129, F13763, H88271, AA342606, AW078502, AI424628, C01861, AI369507, D80045, C14331, D51799, D80253, C14429, D80366, D80391, D59787, D59859, D58283, D81030, D80166, D57483, D80227, D51423, D59619, D80210, D80240, D80196, D80188, D59467, D80212, D59610, D80022, D80043, D80195, D59889, D80219, D80269, D80164, D59275, D59502, D59927, D50995, D80378, D80038, D80241, D80024, C14389, D50979, D80193, AI905856, C75259, C15076, AA305409, AW366296, D51060, AI557751, T03269, AW178893, C14014, D51022, AW179328, AW178775, D80134, D80251, AW375405, AW378532, D51250, AW177440, D80522, AA305578, C14407, D81026, D58253, D80268, AW352158, D80133, F13647, D80248, AA121634, AW369651, D80949, C14227, AW178762, AA514188, Z21582, D80168, D51079, D81111, AA514186, AI910186, AW177501, AW177511, AW375406, AW360811, C05695, C14298, D80064, AI535660, AW352117, AW176467, AW378540, AW377671, AW360844, AW360817, AW378534, AI557241, AW179332, AW377672, AW179023,

				AW178905, D51097, D80132, D80439, AW360834, D59373, T48593, D80302, AA285331, AW352171, AI535686, AW377676, D80247, AW178906, AA809122, AW352170, AW177731, AW178907, AW179019, AW179024, AW177505, AW360841, AW179020, AW178909, AW177456, AW178980, AW179329, AW177733, AW378528, AW178908, AW178754, AW179018, AI536138, AI535639, AI557238, AW179220, AI525856, AW179004, D80157, AW179012, AW178914, AW352174, AW378525, D51103, T11417, D51759, AW177722, AI557808, AW177728, AW179009, AW178774, AW178911, AW378543, AW352163, AW367967, D80014, C06015, AI541027, AW178983, AW352120, D58246, AW178781, D58101, D51002, AI557258, D59653, AI541205, AW177723, D45260, D59503, AI541048, C14344, T03116, AI541321, AI557774, AI557426, D80258, D59627, AL137471, A62298, A62300, AR018138, A84916, Y17188, A82595, AJ132110, X67155, D26022, A25909, A67220, D89785, A78862, D34614, Y17187, X82626, D88547, AF058696, U87250, AR008278, AB028859, A30438, A94995, AR025207, Y11505, X68127, Y12724, S68736, AB012117, A44171, AR016514, U79457, A43190, A85396, AR066482, Y08991, A85477, AR060385, I19525, A86792, AB002449, AR008443, X93549, AF135125, AR008277, AR008281, I50126, I50132, I50128, I50133, AR038669, AR025466, AR066488, AR060138, A45456, A26615, AR052274, Y09669, A43192, AR066487, AR066490, D88507, I14842, AR054175, U46128, D50010, AR050070, I18367, AR016691, AR016690, A63261, A91160, AR008408, AR062872, A70867, AB033111, D13509, A82593, A64136, A68321, AR060133, AR064240, I79511, Z30183, U94592, U87247, and U45328.
9	HTPHS66	48	1042375	R68257, R68551, H88270, H88271, H88539, H88587, H88655, H88699, N63129, W72511, W76069, AA121634, AA584313, AA604083, AA828004, AA961517, AI088690, D81553, C01861, AA292131, AA292132, AA678070, AA704401, AI040077, F13763, AI245928, AI358874, AI347052, AI424628, AI129871, AI243359, AI972342, AW078502, and AW189885.
10	HPJEG57	20	1082921	AI251429, AA535216, AI610468, AA515728, AA825827, AW419389, AI499954, AA570740, AA483606, AI888468, AI187148, AI792575, T74524, AI362442, AI356440, AW191858, AA832145, AI634187, AA568314, AW069227, AW304580, AL079734, AI054398, AA837035,

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11	HHFKM76	52	1042430	AI801477, AI684048, AA442428, AA862447, AI956060, AA532705, AA497001, AI969143, AA100649, AI245777, N99554, AA664442, AI261884, AI220910, AI910540, H06166, AW007144, W69852, AA618024, AI635452, AA999701, AL037618, W69724, T32239, T90870, N71546, AA489586, T95636, Z40505, AA887200, H06165, AI220911, T85871, R51740, D45574, AA090715, AA100648, AW175774, AW149209, AA133885, AA317340, AI476488, AL037619, AA247575, AW079564, Z44649, AA897598, D17227, AC004707, and D17056.
12	HMALI42	22	1047085	N46466.
13	HPJEV11	23	1035601	AI521908, AA972916, AI821981, AA244041, AI951965, AA826002, AF053356, AL121595, AC005482, AL022324, Z96074, AC002528, AC008169, AC006397, AL031287, AC004052, AC004787, Z95325, AC006313, AL032821, AC006379, and L11910.
14	HTTKT43	24	1220693	AI301296, AI076359, and AA971567.
14	HTTKT43	54	1045953	AI076359, and AF152365.
16	HPJDA23	56	1046231	AA582911, AW166815, AA873560, AA522942, AA644207, AI339850, AA523815, AA595825, AA487858, AW080134, AA504871, AA196796, AW104748, AW162049, AI929531, AI133636, AW088202, AL038785, AW029038, AI962050, AI570261, AA486885, AA482711, AW088616, R95704, AI679782, AI251436, AI567674, AA719080, AA620464, AI251002, R91159, AI471481, AA634146, AA713815, AI890923, AW440836, N22018, AA488573, AA480790, AA768905, AA496260, AI963720, AA634145, AL046409, AI917271, AA837084, AI625244, AL120674, AI049722, AI469003, AA111909, AI345418, C06458, U51696, AA115968, AI341664, AI608626, AA550758, AA623002, AA531372, C05755, AI350211, AI368745, AI918421, C06327, AA190911, AA483068, AA767376, AA348929, AI613280, AA630925, T08438, AL119391, F09736, AA099892, AA280632, T94075, AA346458, AA729721, AA192470, AA151980, AA113186, R23229, AA810370, AA159052, AA653618, AA312845, AI283911, AA577906, AA491284, AA521399, AA906889, AA826303, AA490183, AA709005, AA644538, AA132833, AA483223, AI061334, AA644551, AA521323, AA551503, AA708352, AA599920, AA493708, R97934, AA045894, AA923516, AA525824, AA603156, AA970213, AA654771, AA491814, AA649705, AA663486, AA350859, AA806796, AW193432, AA846876, AI344844, AI284640, AA581903, AI708009, AW169537, AA515051, AA601222, AA533725, AA568778,

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17	HTPFX69	59	1042462	AA630332, AI201168, AI937247, AI279435, AW363807, AI951762, AI923289, AI956069, AA769147, AI499201, AA218856, AW245706, AI888174, AI817216, AI983235, AI887469, AW027402, AI922294, AA218917, AI073717, AI064993, AA085946, AI923725, AA602546, AI565024, AA404297, AI812011, AI924074, AW385641, AW090531, AI887792, AI207765, AI223127, AA404254, AA427529, AW384840, AA058823, AI637548, AA404704, AA604366, AA757438, AW392528, AA075582, AI016318, AW385143, AA835526, AA428337, AI754835, AW380207, AI858841, AW008197, AI653455, AA306011, AW247268, AW170338, AL048989, AI951864, AW380198, AW247284, AA478020, AI889825, AA404679, AA689590, AW117973, AI025977, AA633018, AA595997, AA708994, AA425890, AA648361, AA310525, AA430719, AI753602, AA838582, AA189077, AA290670, AI697231, AA845386, AI830847, AI091333, AL119030, AA769478, N21136, AI371226, AA088712, AI291289, AI273324, AI802402, AA983162, AA425962, AI880311, AA479847, AI917890, AI283659, AA633636,

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18	HTFOS57	28	1143810	AA768371, AI721204, AL134524, AL042832, AL045327, AL042420, AL045328, AL134110, AW084068, AI266473, AL047163, AL038878, AL042898, AA515723, AP000208, AP000130, Z99716, AP000247, AC005911, AC005089, AL133445, AL049843, AC003071, AC005015, AC005520, AL121658, AP000359, AL031311, AL132712, AL122021, AP000503, Z93023, AL049569, AC004878, AL079295, AC005102, AL079342, AC002467, AJ246003, AP000496, AC002394, AL035681, AP000355, AL139054, AC005209, Z81369, AB014078, AC004491, AF165926, AL021453, Z83856, AC005736, AF015416, AF134726, AL022302, Z98742, AL022724, AC004938, AC005081, Z99128, Z84466, AC020663, AC005902, Z84469, AL096775, AC006013, AL009181, AP000252, AF095901, AL121825, AP000047, AL034343, AL035658, AC003666, AC007637, AC005484, AL109827, Z93244, AP000031, AL021391, AC005971, AC004832, and AP000011.
18	HTFOS57	61	1043900	AA768371, AI721204, AI266473, AA515723, AA630476, AL133755, Z99716, AP000247, AC005089, AL049835, AP000130, AP000208, AL133445, AC002394, Z93023, AL139054, AC005520, AC005902, AJ246003, AL049843, AL121658, AP000359, AP000074, AC005911, AC003071, AP000503, AC004686, AP000496, AC005015, Z99128, AC020663, AL031311, AL079295, AF134726, AL034343, Z84469, AC002041, AL133245, Z97053, AC005736, AC005081, AL049569, AL050307, AL132712, AP000047, AL022238, AC002467, AP000011, AC002542, Z81369, AC010197, AC005820, AC004983, AC004491, AL096775, AF045555, AC002126, Z86090, AL122021, AC004069, AL035681, AC005067, AC006011, AC005746, AC004099, AP000115, U85195, AL035658, AC007051, AP000355, AC005971, AL024498, AL109798, AL009181, and AC005484.
19	HXOAC69	29	1167171	AI825823, AI700494, AA443455, AI936160, AI884655, AW005803, AI825829, AI700485, AA622020, AI057553, AW044284, AI701135, AW015151, AI828875, AA974971, AI831669, AI923369, AI858013, AI823505, AI251442, AI522289, AI283850, AI857923, AI809046, AW003451, AW025578, AW166494,

				AI200648, AA282170, AI655805, AI337369, AI200543, AI188977, AI686065, AI677688, AW131850, AI439670, AI823504, AW131713, AI656196, AA595124, AA534988, AI478395, AI380902, AI670039, AI089262, AW002239, AI682555, AA873616, AI969193, AI394066, AI199783, AI739075, AA865814, AA448946, H06267, W58413, AI439837, AI478313, AI582728, H06309, AA783023, R50686, AW341798, N94945, AI700886, AI867786, AI624025, H46633, AI206420, R54657, AI744067, N78583, AA765293, AI381330, AA887597, AW044689, AI189570, AI220205, AI288571, H18313, AI916082, W19563, AI940312, H27516, R87847, R33097, H43696, R87404, R50687, AI634986, R87767, R67973, R22237, AA443323, AI868521, AI767139, AA534236, AI985591, AA449673, R83526, AW005824, AI337486, AW028693, R33190, AI823581, R87403, AI735390, AI219632, W58525, H43778, AI962780, AA976289, AI923214, AI352434, AI253727, F32340, AI381329, AA449873, H27586, R66888, AA782805, W07177, AA778050, AA766798, D20444, W23252, AW025883, AA714037, AA973027, AW008884, AI968185, AA935822, AI223201, AW291074, AA021301, AI671294, W72256, AA977192, W58439, AI815580, AW004976, AA435529, AI416961, AW090141, AW169148, R46329, AW237262, AI521080, AI955906, AI147725, W33163, AW081797, AW028196, AI858137, AA199735, Z94789, AW468320, AW770602, and AW772297.
19	HXOAC69	62	1047031	R22237, R33097, R50687, R50686, R54657, R67973, H06267, H27516, H43696, H43778, H46633, R87403, R87404, R87767, N78583, W07177, W19563, W58525, W58413, AA282170, AA534236, AA534988, AA595124, AA622020, AA714037, AA765293, AA766798, AA865814, AA873616, AA887597, AA974971, AA976289, AA443323, AA443455, AA448946, AA449873, AA778050, AA782805, AA783023, AA973027, AI057553, AI089262, D20444, AI251442, AI283850, AI337369, AI352434, AI206420, AI380902, AI394066, AI439837, AI439670, AI478313, AI478395, AI624025, AI188977, AI189570, AI200543, AI200648, AI655805, AI656196, AI219632, AI253727, AI288571, AI337486, AI634986, AI522289, AI671294, AI682555, AI686065, AI739075, AI744067, AI767139, AI700485, AI700494, AI700886, AI701135, AI823505, AI823504, AI825829, AI825823, AI828875, AI831669, AI857923, AI858013, AI884655, AI867786, AI809046, AI916082, AI923214, AI923369, AI936160, AI962780, AI969193, AW003451, AW005803, AW005824, AI985591,

				AW015151, AW025578, AW131713, AW131850, and AW341798.
20	HWMAF61	30	1213297	AI347274, Z99396, AL038837, AL037051, AL036725, AL119324, AL036418, AA631969, AL039074, AL119457, AW392670, AL134524, AL036924, AL039564, AL039085, AL036858, AL039156, AL039108, AL039109, AL039128, AL038509, AL119484, AL119443, AL119399, AW372827, AL037094, AL039659, AW384394, AL038531, AL036196, AL119319, AL119363, AW363220, AL119497, U46349, AL119391, AL039625, AL039648, AL045337, AL119444, AL036767, AL119483, AL042544, AL036190, AL037082, AL037526, AL119355, AL037639, AL039678, AL039629, AL119496, U46350, AL119522, AL039423, AL036238, AL038447, AL134518, AL039150, U46341, AL119418, AL040992, U46351, AL042909, AL119341, AL119335, AL119396, AL134538, AL119464, AL119439, AL037077, AL039386, AL037726, U46347, AL038520, AL036268, AL039410, AL038851, U46346, AL036998, AL037085, AL036733, AL037205, AL037178, AL042984, AL037615, AL042614, AL042965, AL134530, AL036679, AL043029, AL037027, AL042975, AL134528, U46345, AL036191, AL042970, AL134542, AL036719, AL042551, AL134519, AL043033, AL043011, AL043019, AL042450, AL042542, AL036765, AL043003, AL119304, AL036774, AL037021, AL036158, AL036836, AL036999, AL036886, Z12840, AR066494, AR060234, A81671, AR023813, AR064707, AR069079, AR054110, and AB026436.
20	HWMAF61	63	1188597	AI347274.
20	HWMAF61	64	1143835	Z99396, AL038837, AL037051, AL036725, AL036418, AA631969, AL039074, AW392670, AL036924, AL039564, AL039085, AL036858, AL039156, AL039108, AL039109, AL039128, AL038509, AL119484, AW372827, AL037094, AL039659, AW384394, AL038531, AL036196, AL119319, AL119363, AW363220, AL119497, U46349, AL119391, AL039625, AL039648, AL045337, AL119444, AL036767, AL119483, AL036190, AL037082, AL037526, AL119355, AL037639, AL119457, AL119443, AL119324, AL039678, AL039629, AL119496, U46350, AL119522, AL039423, AL036238, AL038447, AL039150, U46341, AL040992, U46351, AL042909, AL119341, AL119335, AL119396, AL134538, AL119439, AL037077, AL119418, AL039386, AL037726, U46347, AL038520, AL134518, AL036268, AL039410, AL038851, U46346, AL036998, AL037085, AL036733, AL037205, AL037178, AL042984, AL037615, AL042614, AL042965, AL134530, AL036679, AL043029, AL037027, AL042975, AL134528,

				AL119399, U46345, AL134524, AL036191, AL042970, AL134542, AL036719, AL042551, AL134519, AL043011, AL042544, AL043019, AL043033, AL042450, AL042542, AL036765, AL043003, AL119304, AL119464, AL036774, AL037021, AL036158, AL036836, AL036999, AL036886, AL036964, Z12840, AR060234, AR066494, A81671, AR023813, AR064707, AR069079, AB026436, and AR054110.
20	HWMAF61	65	1033715	Z12840.
20	HWMAF61	66	1047027	AI347274.
21	HMSOC30	31	1033846	AA158537, AI002979, AA806165, AI702990, AI091292, AI005391, N54188, AA069178, AA091807, AA095638, and AC006146.
21	HMSOC30	67	1045945	AI525927, and AC006146.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

5

Examples

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector.

10 Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being

15 isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited Plasmid</u>
	Lambda Zap	pBluescript (pBS)
	Uni-Zap XR	pBluescript (pBS)
20	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
	pCMVSPORT 3.0	pCMVSPORT 3.0
25	pCR [®] 2.1	pCR [®] 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res.

30 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey

Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3
5 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

10 Vectors pSport1, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University,
15 NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR[®]2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al.,
20 Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional
25 plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample
30 may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

- 5 Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P - γ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).)
- 10 The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate.
- 15 These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

- Alternatively, two primers of 17-20 nucleotides derived from both ends of the
- 20 SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 μl of reaction mixture with 0.5 μg of the above cDNA template. A convenient reaction mixture is
- 25 1.5-5 mM MgCl_2 , 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and
- 30 the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A⁺ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

Example 3: Tissue Distribution of Polypeptide

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P^{32} using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers
5 used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a
10 bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at
15 the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and
20 confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG
25 (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic
30 agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from

QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QLAexpressionist (1995) QIAGEN, Inc., *supra*).

5 Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl.
10 Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250
15 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements
20 operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains:
1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The
25 origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA
30 insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or

Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

5

Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

10 Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution
15 containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

 The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by
20 centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

 The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree
25 C overnight to allow further GuHCl extraction.

 Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C
30 without mixing for 12 hours prior to further purification steps.

 To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area

(e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a
5 stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak
10 anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are
15 collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified
20 protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus

25 Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as
30 BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak

Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

5 Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-
10 39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does
15 not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

20 The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and
25 optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue
30 (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by

gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™
5 baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are
10 added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf
15 serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of
20 a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the
25 suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with
30 the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and

cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of ^{35}S -methionine and 5 uCi ^{35}S -cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by
5 SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

10 The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening
15 sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

20 Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1,
25 Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification
30 and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment

then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector
5 are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μ g of the expression plasmid pC6 a pC4 is cotransfected with 0.5
10 μ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in
15 alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing
20 even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

25 **Example 9: Protein Fusions**

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see
30 also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a

specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by
5 modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These
10 primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with
15 BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted
20 protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

25 GGGATCCGGAGCCCAAATCTTCTGACAAAACCTCACACATGCCCACC
GTGCCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCC
AAAACCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCG
TGGTGGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
30 AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT
CCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA

GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAA
CCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC
GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCAC
5 CGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGA
TGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT
CCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

Example 10: Production of an Antibody from a Polypeptide

10 The antibodies of the present invention can be prepared by a variety of
methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells
expressing a polypeptide of the present invention is administered to an animal to
induce the production of sera containing polyclonal antibodies. In a preferred
method, a preparation of the secreted protein is prepared and purified to render it
15 substantially free of natural contaminants. Such a preparation is then introduced into
an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are
monoclonal antibodies (or protein binding fragments thereof). Such monoclonal
antibodies can be prepared using hybridoma technology. (Köhler et al.,
20 Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al.,
Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-
Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures
involve immunizing an animal (preferably a mouse) with polypeptide or, more
preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in
25 any suitable tissue culture medium; however, it is preferable to culture cells in Earle's
modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at
about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids,
about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma
30 cell line. Any suitable myeloma cell line may be employed in accordance with the
present invention; however, it is preferable to employ the parent myeloma cell line
(SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are

selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

5 Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a
10 mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of
15 further protein-specific antibodies.

It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂
20 fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced
25 using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson
30 et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Example 11: Production Of Secreted Protein For High-Throughput Screening Assays

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described herein.

5 First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel).

10 Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2×10^5 cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine
15 (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an
20 expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem
25 I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates
30 of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells

first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl₂ (anhyd); 0.00130 mg/L CuSO₄·5H₂O; 0.050 mg/L of Fe(NO₃)₃·9H₂O; 0.417 mg/L of FeSO₄·7H₂O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl₂; 48.84 mg/L of MgSO₄; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO₃; 62.50 mg/L of NaH₂PO₄·H₂O; 71.02 mg/L of Na₂HPO₄; .4320 mg/L of ZnSO₄·7H₂O; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H₂O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H₂O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H₂O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H₂O; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B₁₂; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L

DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person
5 B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

10 It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant
15 characterized by an activity in a particular assay.

Example 12: Construction of GAS Reporter Construct

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the
20 Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six
25 members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in
30 tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are
5 generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-
10 12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN- α , IFN- γ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn
15 activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines
20 are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

<u>JAKs</u>					<u>STATs</u>	<u>GAS(elements) or ISRE</u>
<u>Ligand</u>		<u>tyk2</u>	<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>	
<u>IFN family</u>						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g			+	+	-	1
IL-10		+	?	?	-	1,3
<u>gp130 family</u>						
IL-6 (Pleiotrophic)		+	+	+	?	1,3
IL-11(Pleiotrophic)		?	+	?	?	1,3
OnM(Pleiotrophic)		?	+	+	?	1,3
LIF(Pleiotrophic) ?		+	+	?	1,3	
CNTF(Pleiotrophic)	-/+	+	+	?	1,3	
G-CSF(Pleiotrophic)	?	+	?	?	1,3	
IL-12(Pleiotrophic)	+	-	+	+	1,3	
<u>g-C family</u>						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >> Ly6)(IgH)
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
<u>gp140 family</u>						
IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
IL-5 (myeloid)	-	-	+	-	5	GAS
GM-CSF (myeloid)	-	-	+	-	5	GAS
<u>Growth hormone family</u>						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
<u>Receptor Tyrosine Kinases</u>						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

10 5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTT
CCCCGAAATGATTTCCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID
NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3'
15 (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following
20 sequence:

5':CTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCC
GAAATGATTTCCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGT
CCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCA
TTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGC
25 CGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAG
GCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be
30 instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and
5 XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-
10 SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding
15 as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described
20 in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

25

Example 13: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the
30 GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152),

although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4⁺ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-
5 SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is
10 demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1% Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life
15 Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1×10^7 cells in OPTI-MEM to T25
20 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptides of the invention and/or induced polypeptides of
25 the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100
30 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred
5 directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed
10 in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve
15 as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as,
20 stable transfected cells, which would be apparent to those of skill in the art.

Example 14: High-Throughput Screening Assay Identifying Myeloid Activity

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells.
25 Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct
30 produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2×10^7 U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing

10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM
5 KCl, 375 uM Na₂HPO₄·7H₂O, 1 mM MgCl₂, and 675 uM CaCl₂. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400
10 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting 1×10^8 cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of 5×10^5 cells/ml. Plate 200 ul cells per well in
15 the 96-well plate (or 1×10^5 cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the
20 supernatant according to the protocol described in Example 17.

Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes,
25 EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or
30 differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably

transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene
5 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified
10 product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30
15 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-
20 inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine
25 protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80%
30 confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5×10^5 cells/ml.

- 5 Add 200 μ l of the cell suspension to each well of 96-well plate (equivalent to 1×10^5 cells/well). Add 50 μ l supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ μ l of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the
10 supernatant according to Example 17.

Example 16: High-Throughput Screening Assay for T-cell Activity

- NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and
15 CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

- 20 In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

- 25 Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid
30 arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-

KB binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCC
5 GGGACTTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

10 PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

15 5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC
TTTCCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTC
CGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATG
GCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTG
AGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTTGGAGGCCTAGGCTTTTGC
20 AAAAAGCTT:3' (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and
25 therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the
30 GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly,

the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

5 **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

10 Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

15 Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at
20 each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation:

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5

21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small

molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star
5 black 96-well plate with clear bottom. The plate is incubated in a CO₂ incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To
load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate
10 is incubated at 37 degrees C in a CO₂ incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are
re-suspended to 2-5x10⁶ cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml
fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension.
15 The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are
washed twice with HBSS, resuspended to 1x10⁶ cells/ml, and dispensed into a
microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is
then washed once in Denley CellWash with 200 ul, followed by an aspiration step to
100 ul final volume.

20 For a non-cell based assay, each well contains a fluorescent molecule, such as
fluo-4. The supernatant is added to the well, and a change in fluorescence is
detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the
following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4
25 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm;
and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an
extracellular signaling event which has resulted in an increase in the intracellular
Ca⁺⁺ concentration.

30 **Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase
Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen

Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyn plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na₃VO₄, 2 mM Na₄P₂O₇ and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg₂⁺ (5mM ATP/50mM MgCl₂), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl₂, 5 mM MnCl₂, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the

components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mm EDTA and place the reactions on ice.

5 Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish
10 peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound
15 peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity

20 As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other
25 molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA
30 plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against

Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

5 A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

10 After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard
15 procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

20 **Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide**

 RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA
25 is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

 PCR products are then sequenced using primers labeled at their 5' end with T4
30 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring

suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

5 PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

10 Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

15 Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and
20 chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

25

Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

30 A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 23: Formulation

The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual

patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

5 As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for
10 the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears
15 to vary depending on the desired effect.

Therapeutics can be are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray.

"Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid
20 filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-
25 release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or
30 formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials
5 (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al.,
10 J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (see generally, Langer, *Science* 249:1527-1533 (1990);
15 Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP
20 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

25 In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer
30 (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage

injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container

having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized
5 formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or
10 more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in
15 conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG
20 (e.g., THERACYS®), MPL and nonviable preparations of *Corynebacterium parvum*. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to,
25 Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Viroosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B,
30 whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture,

separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or therapeutic treatments described below. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™

(ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott; COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEAPp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'-azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β -L-FD4C and β -L-FddC (WO 98/17281).

Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR™

(PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity
5 against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Wellcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the
10 HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4
15 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists.
20 Chemokine receptor agonists such as RANTES, SDF-1, MIP-1 α , MIP-1 β , etc., may also inhibit fusion.

Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones;
25 ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide
30 reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors such as VX-497 (Vertex); and mycopholic acids such as CellCept (mycophenolate mofetil; Roche).

Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and
5 AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1 α , MIP-1 β , SDF-1 α , IL-2, PROLEUKINTM (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as
10 IFN- α 2a; antagonists of TNFs, NF κ B, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as RemuneTM (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched
15 synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al.*, *PNAS* 94:11567-72 (1997); Chen *et al.*,
20 *Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF- α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor
25 agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α -naphthoflavone (WO 98/30213); and antioxidants such as γ -L-glutamyl-L-cysteine ethyl ester (γ -GCE; WO 99/56764).

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered
30 with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with

PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial
5 infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases,
10 Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rapamycin, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

In other embodiments, Therapeutics of the invention are administered in
15 combination with immunosuppressive agents. Immunosuppressive agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.
20 Other immunosuppressive agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685), ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™,
25 SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate mofetil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucocorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrxate), OXSORALEN-ULTRA™ (methoxsalen) and
30 RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™ (antithymocyte globulin), and GAMMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

10 In certain embodiments, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, corticosteroids (e.g. betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (e.g., diclofenac, diflunisal, etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tenoxicam, tiaprofenic acid, and tolmetin.), as well as antihistamines, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent. Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor

of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition
5 metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium
10 metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten
15 oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its
20 hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and
25 sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, (1991)); Sulphated
30 Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline

analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, (1992)); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, (1990)); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrone (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, (1992)); and metalloproteinase inhibitors such as BB94.

Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolimidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

Anti-angiogenic agents that may be administed in combination with the compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic

inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the compositions of the invention include, but are not limited to, EMD-121974 (Merck KgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

In another embodiment, the polynucleotides encoding a polypeptide of the present invention are administered in combination with an angiogenic protein, or polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins that may be administered with the compositions of the invention include, but are not
5 limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin-like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide
10 synthase.

In additional embodiments, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to alkylating agents such as nitrogen mustards (for example, Mechlorethamine,
15 cyclophosphamide, Cyclophosphamide Ifosfamide, Melphalan (L-sarcolysin), and Chlorambucil), ethylenimines and methylmelamines (for example, Hexamethylmelamine and Thiotepa), alkyl sulfonates (for example, Busulfan), nitrosoureas (for example, Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU), and Streptozocin (streptozotocin)), triazenes (for example,
20 Dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide)), folic acid analogs (for example, Methotrexate (amethopterin)), pyrimidine analogs (for example, Fluorouracil (5-fluorouracil; 5-FU), Floxuridine (fluorodeoxyuridine; FudR), and Cytarabine (cytosine arabinoside)), purine analogs and related inhibitors (for example, Mercaptopurine (6-mercaptopurine; 6-MP), Thioguanine (6-thioguanine;
25 TG), and Pentostatin (2'-deoxycoformycin)), vinca alkaloids (for example, Vinblastine (VLB, vinblastine sulfate)) and Vincristine (vincristine sulfate)), epipodophyllotoxins (for example, Etoposide and Teniposide), antibiotics (for example, Dactinomycin (actinomycin D), Daunorubicin (daunomycin; rubidomycin), Doxorubicin, Bleomycin, Plicamycin (mithramycin), and Mitomycin (mitomycin C),
30 enzymes (for example, L-Asparaginase), biological response modifiers (for example, Interferon-alpha and interferon-alpha-2b), platinum coordination compounds (for example, Cisplatin (cis-DDP) and Carboplatin), anthracenedione (Mitoxantrone),

substituted ureas (for example, Hydroxyurea), methylhydrazine derivatives (for example, Procarbazine (N-methylhydrazine; MII), adrenocorticosteroids (for example, Prednisone), progestins (for example, Hydroxyprogesterone caproate, Medroxyprogesterone, Medroxyprogesterone acetate, and Megestrol acetate),
5 estrogens (for example, Diethylstilbestrol (DES), Diethylstilbestrol diphosphate, Estradiol, and Ethinyl estradiol), antiestrogens (for example, Tamoxifen), androgens (Testosterone propionate, and Fluoxymesterone), antiandrogens (for example, Flutamide), gonadotropin-releasing hormone analogs (for example, Leuprolide), other hormones and hormone analogs (for example, methyltestosterone, estramustine,
10 estramustine phosphate sodium, chlorotrianisene, and testolactone), and others (for example, dicarbazine, glutamic acid, and mitotane).

In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also
15 known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.)

In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one
20 embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or
25 prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the
30 invention are administered in combination with tositumomab. In a further embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of

CHOP, particularly cyclophosphamide and/or prednisone. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more radisotopes. Particularly preferred isotopes are ⁹⁰Y and ¹¹¹In.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number
5 EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993);
10 Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth
15 Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent
20 Number DE19639601. The above mentioned references are herein incorporated by reference in their entireties.

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but
25 are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention
30 include, but are not limited to, granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim, LEUKINE™, PROKINE™), granulocyte colony stimulating factor (G-CSF) (filgrastim, NEUPOGEN™), macrophage colony

stimulating factor (M-CSF, CSF-1) erythropoietin (epoetin alfa, EPOGEN™, PROCRIT™), stem cell factor (SCF, c-kit ligand, steel factor), megakaryocyte colony stimulating factor, PIXY321 (a GMCSF/IL-3 fusion protein), interleukins, especially any one or more of IL-1 through IL-12, interferon-gamma, or
5 thrombopoietin.

In certain embodiments, Therapeutics of the present invention are administered in combination with adrenergic blockers, such as, for example, acebutolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, and timolol.

10 In another embodiment, the Therapeutics of the invention are administered in combination with an antiarrhythmic drug (e.g., adenosine, amiodarone, bretylium, digitalis, digoxin, digitoxin, diltiazem, disopyramide, esmolol, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, N-acetyl procainamide, propafenone, propranolol, quinidine, sotalol, tocainide, and verapamil).

15 In another embodiment, the Therapeutics of the invention are administered in combination with diuretic agents, such as carbonic anhydrase-inhibiting agents (e.g., acetazolamide, dichlorphenamide, and methazolamide), osmotic diuretics (e.g., glycerin, isosorbide, mannitol, and urea), diuretics that inhibit $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ symport (e.g., furosemide, bumetanide, azosemide, piretanide, tripamide, ethacrynic acid, muzolimine, and torsemide), thiazide and thiazide-like diuretics (e.g., bendroflumethiazide, benzthiazide, chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichormethiazide, chlorthalidone, indapamide, metolazone, and quinethazone), potassium sparing
20 diuretics (e.g., amiloride and triamterene), and mineralcorticoid receptor antagonists (e.g., spironolactone, canrenone, and potassium canrenoate).
25

In one embodiment, the Therapeutics of the invention are administered in combination with treatments for endocrine and/or hormone imbalance disorders. Treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, ^{127}I , radioactive isotopes of iodine such as ^{131}I and ^{123}I ; recombinant
30 growth hormone, such as HUMATROPE™ (recombinant somatropin); growth hormone analogs such as PROTROPIN™ (somatrem); dopamine agonists such as PARLODEL™ (bromocriptine); somatostatin analogs such as SANDOSTATIN™

(octreotide); gonadotropin preparations such as PREGNYL™, A.P.L.™ and PROFASI™ (chorionic gonadotropin (CG)), PERGONAL™ (menotropins), and METRODIN™ (urofollitropin (uFSH)); synthetic human gonadotropin releasing hormone preparations such as FACTREL™ and LUTREPULSE™ (gonadorelin hydrochloride); synthetic gonadotropin agonists such as LUPRON™ (leuprolide acetate), SUPPRELIN™ (histrelin acetate), SYNAREL™ (nafarelin acetate), and ZOLADEX™ (goserelin acetate); synthetic preparations of thyrotropin-releasing hormone such as RELEFACT TRH™ and THYPINONE™ (protirelin); recombinant human TSH such as THYROGEN™; synthetic preparations of the sodium salts of the natural isomers of thyroid hormones such as L-T₄™, SYNTHROID™ and LEVOTHROID™ (levothyroxine sodium), L-T₃™, CYTOMEL™ and TRIOSTAT™ (liothyroine sodium), and THYROLAR™ (liotrix); antithyroid compounds such as 6-*n*-propylthiouracil (propylthiouracil), 1-methyl-2-mercaptoimidazole and TAPAZOLE™ (methimazole), NEO-MERCAZOLE™ (carbimazole); beta-adrenergic receptor antagonists such as propranolol and esmolol; Ca²⁺ channel blockers; dexamethasone and iodinated radiological contrast agents such as TELEPAQUE™ (iopanoic acid) and ORAGRAFIN™ (sodium ipodate).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, estrogens or conjugated estrogens such as ESTRACE™ (estradiol), ESTINYL™ (ethinyl estradiol), PREMARIN™, ESTRATAB™, ORTHO-EST™, OGEN™ and estropipate (estrone), ESTROVIS™ (quinestrol), ESTRADERM™ (estradiol), DELESTROGEN™ and VALERGEN™ (estradiol valerate), DEPO-ESTRADIOL CYPIONATE™ and ESTROJECT LA™ (estradiol cypionate); antiestrogens such as NOLVADEX™ (tamoxifen), SEROPHENE™ and CLOMID™ (clomiphene); progestins such as DURALUTIN™ (hydroxyprogesterone caproate), MPA™ and DEPO-PROVERA™ (medroxyprogesterone acetate), PROVERA™ and CYCRIN™ (MPA), MEGACE™ (megestrol acetate), NORLUTIN™ (norethindrone), and NORLUTATE™ and AYGESTIN™ (norethindrone acetate); progesterone implants such as NORPLANT SYSTEM™ (subdermal implants of norgestrel); antiprogestins such as RU 486™

(mifepristone); hormonal contraceptives such as ENOVID™ (norethynodrel plus mestranol), PROGESTASERT™ (intrauterine device that releases progesterone), LOESTRIN™, BREVICON™, MODICON™, GENORA™, NELONA™, NORINYL™, OVACON-35™ and OVACON-50™ (ethinyl estradiol/norethindrone),
5 LEVLEN™, NORDETTE™, TRI-LEVLEN™ and TRIPHASIL-21™ (ethinyl estradiol/levonorgestrel) LO/OVRAL™ and OVRAL™ (ethinyl estradiol/norgestrel), DEMULEN™ (ethinyl estradiol/ethynodiol diacetate), NORINYL™, ORTHO-NOVUM™, NORETHIN™, GENORA™, and NELOVA™ (norethindrone/mestranol), DESOGEN™ and ORTHO-CEPT™ (ethinyl estradiol/desogestrel), ORTHO-
10 CYCLEN™ and ORTHO-TRICYCLEN™ (ethinyl estradiol/norgestimate), MICRONOR™ and NOR-QD™ (norethindrone), and OVRETTE™ (norgestrel).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, testosterone esters such as methenolone acetate and testosterone undecanoate; parenteral and oral androgens such as TESTOJECT-50™
15 (testosterone), TESTEX™ (testosterone propionate), DELATESTRYL™ (testosterone enanthate), DEPO-TESTOSTERONE™ (testosterone cypionate), DANOCRINE™ (danazol), HALOTESTIN™ (fluoxymesterone), ORETON METHYL™, TESTRED™ and VIRILON™ (methyltestosterone), and OXANDRIN™ (oxandrolone); testosterone transdermal systems such as TESTODERM™; androgen receptor
20 antagonist and 5-alpha-reductase inhibitors such as ANDROCUR™ (cyproterone acetate), EULEXIN™ (flutamide), and PROSCAR™ (finasteride); adrenocorticotrophic hormone preparations such as CORTROSYN™ (cosyntropin); adrenocortical steroids and their synthetic analogs such as ACLOVATE™ (alclometasone dipropionate), CYCLOCORT™ (amcinonide), BECLOVENT™ and VANCERIL™ (beclomethasone
25 dipropionate), CELESTONE™ (betamethasone), BENISONE™ and UTICORT™ (betamethasone benzoate), DIPROSONE™ (betamethasone dipropionate), CELESTONE PHOSPHATE™ (betamethasone sodium phosphate), CELESTONE SOLUSPAN™ (betamethasone sodium phosphate and acetate), BETA-VAL™ and VALISONE™ (betamethasone valerate), TEMOVATE™ (clobetasol propionate),
30 CLODERM™ (clocortolone pivalate), CORTEF™ and HYDROCORTONE™

(cortisol (hydrocortisone)), HYDROCORTONE ACETATE™ (cortisol (hydrocortisone) acetate), LOCOID™ (cortisol (hydrocortisone) butyrate), HYDROCORTONE PHOSPHATE™ (cortisol (hydrocortisone) sodium phosphate), A-HYDROCORT™ and SOLU CORTEF™ (cortisol (hydrocortisone) sodium succinate), WESTCORT™ (cortisol (hydrocortisone) valerate), CORTISONE ACETATE™ (cortisone acetate), DESOWEN™ and TRIDESILON™ (desonide), TOPICORT™ (desoximetasone), DECADRON™ (dexamethasone), DECADRON LA™ (dexamethasone acetate), DECADRON PHOSPHATE™ and HEXADROL PHOSPHATE™ (dexamethasone sodium phosphate), FLORONE™ and
10 MAXIFLOR™ (diflorasone diacetate), FLORINEF ACETATE™ (fludrocortisone acetate), AEROBID™ and NASALIDE™ (flunisolide), FLUONID™ and SYNALAR™ (fluocinolone acetonide), LIDEX™ (fluocinonide), FLUOR-OP™ and FML™ (fluorometholone), CORDRAN™ (flurandrenolide), HALOG™ (halcinonide), HMS LIZUIFILM™ (medrysone), MEDROL™ (methylprednisolone), DEPO-
15 MEDROL™ and MEDROL ACETATE™ (methylprednisone acetate), A-METHAPRED™ and SOLUMEDROL™ (methylprednisolone sodium succinate), ELOCON™ (mometasone furoate), HALDRONE™ (paramethasone acetate), DELTA-CORTEF™ (prednisolone), ECONOPRED™ (prednisolone acetate), HYDELTRASOL™ (prednisolone sodium phosphate), HYDELTRA-T.B.A™
20 (prednisolone tebutate), DELTASONE™ (prednisone), ARISTOCORT™ and KENACORT™ (triamcinolone), KENALOG™ (triamcinolone acetonide), ARISTOCORT™ and KENACORT DIACETATE™ (triamcinolone diacetate), and ARISTOSPAN™ (triamcinolone hexacetonide); inhibitors of biosynthesis and action of adrenocortical steroids such as CYTADREN™ (aminoglutethimide), NIZORAL™
25 (ketoconazole), MODRASTANE™ (trilostane), and METOPIRONE™ (metyrapone); bovine, porcine or human insulin or mixtures thereof; insulin analogs; recombinant human insulin such as HUMULIN™ and NOVOLIN™; oral hypoglycemic agents such as ORAMIDE™ and ORINASE™ (tolbutamide), DIABINESE™
(chlorpropamide), TOLAMIDE™ and TOLINASE™ (tolazamide), DYMELOR™
30 (acetoexamide), glibenclamide, MICRONASE™, DIBETA™ and GLYNASE™

(glyburide), GLUCOTROL™ (glipizide), and DIAMICRON™ (gliclazide), GLUCOPHAGE™ (metformin), ciglitazone, pioglitazone, and alpha-glucosidase inhibitors; bovine or porcine glucagon; somatostatins such as SANDOSTATIN™ (octreotide); and diazoxides such as PROGLYCEM™ (diazoxide).

5 In one embodiment, the Therapeutics of the invention are administered in combination with treatments for uterine motility disorders. Treatments for uterine motility disorders include, but are not limited to, estrogen drugs such as conjugated estrogens (e.g., PREMARIN® and ESTRATAB®), estradiols (e.g., CLIMARA® and ALORA®), estropipate, and chlorotrianisene; progestin drugs (e.g., AMEN®
10 (medroxyprogesterone), MICRONOR® (norethidrone acetate), PROMETRIUM® progesterone, and megestrol acetate); and estrogen/progesterone combination therapies such as, for example, conjugated estrogens/medroxyprogesterone (e.g., PREMPRO™ and PREMPHASE®) and norethindrone acetate/ethinyl estsradiol (e.g., FEMHRT™).

15 In an additional embodiment, the Therapeutics of the invention are administered in combination with drugs effective in treating iron deficiency and hypochromic anemias, including but not limited to, ferrous sulfate (iron sulfate, FEOSOL™), ferrous fumarate (e.g., FEOSTAT™), ferrous gluconate (e.g., FERGON™), polysaccharide-iron complex (e.g., NIFEREX™), iron dextran
20 injection (e.g., INFED™), cupric sulfate, pyroxidine, riboflavin, Vitamin B₁₂, cyancobalamin injection (e.g., REDISOL™, RUBRAMIN PC™), hydroxocobalamin, folic acid (e.g., FOLVITE™), leucovorin (folinic acid, 5-CHOH4PteGlu, citrovorum factor) or WELLCOVORIN (Calcium salt of leucovorin), transferrin or ferritin.

 In certain embodiments, the Therapeutics of the invention are administered in
25 combination with agents used to treat psychiatric disorders. Psychiatric drugs that may be administered with the Therapeutics of the invention include, but are not limited to, antipsychotic agents (e.g., chlorpromazine, chlorprothixene, clozapine, fluphenazine, haloperidol, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, quetiapine, risperidone, thioridazine, thiothixene,
30 trifluoperazine, and triflupromazine), antimanic agents (e.g., carbamazepine, divalproex sodium, lithium carbonate, and lithium citrate), antidepressants (e.g.,

amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, fluoxetine, imipramine, isocarboxazid, maprotiline, mirtazapine, nefazodone, nortriptyline, paroxetine, phenelzine, protriptyline, sertraline, tranylcypromine, trazodone, trimipramine, and venlafaxine), antianxiety
5 agents (e.g., alprazolam, buspirone, chlordiazepoxide, clorazepate, diazepam, halazepam, lorazepam, oxazepam, and prazepam), and stimulants (e.g., d-amphetamine, methylphenidate, and pemoline).

In other embodiments, the Therapeutics of the invention are administered in combination with agents used to treat neurological disorders. Neurological agents
10 that may be administered with the Therapeutics of the invention include, but are not limited to, antiepileptic agents (e.g., carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid, divalproex sodium, felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, zonisamide, diazepam, lorazepam, and clonazepam), antiparkinsonian agents (e.g.,
15 levodopa/carbidopa, selegiline, amantidine, bromocriptine, pergolide, ropinirole, pramipexole, benztropine; biperiden; ethopropazine; procyclidine; trihexyphenidyl, tolcapone), and ALS therapeutics (e.g. riluzole).

In another embodiment, Therapeutics of the invention are administered in combination with vasodilating agents and/or calcium channel blocking agents.
20 Vasodilating agents that may be administered with the Therapeutics of the invention include, but are not limited to, Angiotensin Converting Enzyme (ACE) inhibitors (e.g., papaverine, isoxsuprine, benazepril, captopril, cilazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril,trandolapril, and nylidrin), and nitrates (e.g., isosorbide dinitrate, isosorbide mononitrate, and
25 nitroglycerin). Examples of calcium channel blocking agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to amlodipine, bepridil, diltiazem, felodipine, flunarizine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil.

In additional embodiments, the Therapeutics of the invention are administered
30 in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 24: Method of Treating Decreased Levels of the Polypeptide

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

Example 25: Method of Treating Increased Levels of the Polypeptide

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

Example 26: Method of Treatment Using Gene Therapy-Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and
5 separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS,
10 penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

15 pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified
20 using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are
25 added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

30 The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the

gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

5 Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. 10 If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced. 15 The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention

20 Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, 25 published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

30 Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be

operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction
5 enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal
10 phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

15 In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

20 Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

25 Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is
30 resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂ HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1

mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3×10^6 cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to
5 construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one
10 non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-
15 digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 $\mu\text{g/ml}$. 0.5 ml of the cell suspension (containing approximately 1.5×10^6 cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is
20 performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

25 Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a
30 further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts

now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 28: Method of Treatment Using Gene Therapy - In Vivo

5 Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter
10 or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318
15 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The
20 polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or
25 precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

30 The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in

the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to
5 provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and
10 connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of
15 the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely
20 differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg
25 body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the
30 condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an

aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is
5 determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

10 Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the
15 knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A
20 time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper
25 dosages and other treatment parameters in humans and other animals using naked DNA.

Example 29: Transgenic Animals.

The polypeptides of the invention can also be expressed in transgenic animals.
30 Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a

specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the

particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the

transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 30: Knock-Out Animals.

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (*i.e.*,

animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc.

The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 31: Production of an Antibody**Hybridoma Technology**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide(s) of the invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide(s) of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide(s) of the invention are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide(s) of the invention, or, more preferably, with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide(s) of the invention.

Alternatively, additional antibodies capable of binding polypeptide(s) of the invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and

therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones
5 which produce an antibody whose ability to bind to the polypeptide(s) of the invention protein-specific antibody can be blocked by polypeptide(s) of the invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide(s) of the invention protein-specific antibody and are used to immunize an animal to induce formation of further polypeptide(s) of the invention protein-specific antibodies.

10 For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214
15 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

20 *Isolation Of Antibody Fragments Directed polypeptide(s) of the invention From A Library Of scFvs*

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the invention to which the donor may or may not have been exposed (see e.g., U.S. Patent
25 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10⁹ E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin
30 (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10⁸ TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture

incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication
5 WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative
10 supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and
15 concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10¹³ transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS
20 with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10¹³ TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20
25 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose
30 and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification

with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the

detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3 H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell

representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+,
5 CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the
10 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 33: T Cell Proliferation Assay

15 Proliferation assay for Resting PBLs.

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^3H -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 microliters per well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 °C (1 microgram/ml in .05M bicarbonate buffer, pH 9.5), then wash
20 three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5×10^4 /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of TNF Delta and/or TNF Epsilon protein (total volume 200 microliters). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 °C, plates are spun for 2 min. at 1000 rpm and 100 microliters of
25 supernatant is removed and stored -20 °C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 microliters of medium containing 0 microcuries of ^3H -thymidine and cultured at 37 °C for 18-24 hr. Wells are harvested and incorporation of ^3H -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation
30 Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TNF Delta and/or TNF Epsilon proteins.

Alternatively, a proliferation assay on resting PBL (peripheral blood lymphocytes) is measured by the up-take of ^3H -thymidine. The assay is performed as follows. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non-adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2×10^4 cells/well in a final volume of 200 microliters. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2 (*), IFN γ , TNF α , IL-10 and TR2. In addition to the control supernatants, recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 100ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^3H -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^3H -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

(*) The amount of the control cytokines IL-2, IFN γ , TNF α and IL-10 produced in each transfection varies between 300pg to 5ng/ml.

Costimulation assay.

A costimulation assay on resting PBL (peripheral blood lymphocytes) is performed in the presence of immobilized antibodies to CD3 and CD28. The use of antibodies specific for the invariant regions of CD3 mimic the induction of T cell activation that would occur through stimulation of the T cell receptor by an antigen. Cross-linking of the TCR (first signal) in the absence of a costimulatory signal (second signal) causes very low induction of proliferation and will eventually result in a state of "anergy", which is characterized by the absence of growth and inability to

produce cytokines. The addition of a costimulatory signal such as an antibody to CD28, which mimics the action of the costimulatory molecule. B7-1 expressed on activated APCs, results in enhancement of T cell responses including cell survival and production of IL-2. Therefore this type of assay allows to detect both positive and negative effects caused by addition of supernatants expressing the proteins of interest on T cell proliferation.

The assay is performed as follows. Ninety-six well plates are coated with 100ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 100ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2×10^4 cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only (negative control), IL-2, IFN γ , TNF α , IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^3H -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^3H -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

Costimulation assay: IFN γ and IL-2 ELISA.

The assay is performed as follows. Twenty-four well plates are coated with either 300ng/ml or 600ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San

Diego, CA) in a final volume of 500ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the costimulation assay. The assay is performed in the pre-coated twenty-four well plate using 1×10^5 cells/well in a final volume of 900ul. The supernatants (293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 300ul are added to 600ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only(negative control), IL-2, IFN γ , IL-12 and IL-18. In addition to the control supernatants recombinant human IL-2 (all cytokines were purchased from R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml, IL-12 at a final concentration of 1ng/ml and IL-18 at a final concentration of 50ng/ml are also used. Controls and unknown samples are tested in duplicate. Supernatant samples (250ul) are collected 2 days and 5 days after the beginning of the assay. ELISAs to test for IFN γ and IL-2 secretion are performed using kits purchased from R & D Systems, (Minneapolis, MN). Results are expressed as an average of duplicate samples plus or minus standard error.

Proliferation assay for preactivated-resting T cells.

A proliferation assay on preactivated-resting T cells is performed on cells that are previously activated with the lectin phytohemagglutinin (PHA). Lectins are polymeric plant proteins that can bind to residues on T cell surface glycoproteins including the TCR and act as polyclonal activators. PBLs treated with PHA and then cultured in the presence of low doses of IL-2 resemble effector T cells. These cells are generally more sensitive to further activation induced by growth factors such as IL-2. This is due to the expression of high affinity IL-2 receptors that allows this population to respond to amounts of IL-2 that are 100 fold lower than what would

have an effect on a naïve T cell. Therefore the use of this type of cells might enable to detect the effect of very low doses of an unknown growth factor, that would not be sufficient to induce proliferation on resting (naïve) T cells.

The assay is performed as follows. PBMC are isolated by F/H gradient
5 centrifugation from human peripheral blood, and are cultured in 10% FCS (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD) in the presence of 2 µg/ml PHA (Sigma, Saint Louis, MO) for three days. The cells are then washed in PBS and cultured in 10% FCS/RPMI in the presence of 5 ng/ml of human recombinant IL-2 (R & D Systems, Minneapolis, MN) for 3 days. The cells are
10 washed and rested in starvation medium (1% FCS/RPMI) for 16 hours prior to the beginning of the proliferation assay. An aliquot of the cells is analyzed by FACS to determine the percentage of T cells (CD3 positive cells) present; this usually ranges between 93-97% depending on the donor. The assay is performed in a 96 well plate using 2×10^4 cells/well in a final volume of 200 µl. The supernatants (e.g., CHO or
15 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60 µl are added to 140 µl of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2, IFN , TNF , IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 at a final concentration of 10 ng/ml is
20 also used. After 24 hours of culture, each well is pulsed with 1 µCi of ^3H -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^3H -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

The studies described in this example test activity of polypeptides of the
25 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells

5 Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- α , causes a rapid
10 change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC γ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3
15 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

20

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to
25 measure the IL-12 release as follows. Dendritic cells (10^6 /ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with
30 the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other
5 costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes
10 are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

15 Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or
20 activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal
25 elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating
30 factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free

medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2×10^6 /ml in PBS containing PI at a final concentration of 5 μ g/ml, and then incubated at room temperature for 5 minutes before
5 FACSscan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through
10 the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the
15 invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at 2×10^5
20 cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol
25 red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 μ l 1N NaOH per well. The absorbance is read at 610
30 nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies

to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 35: Biological Effects of Polypeptides of the Invention

5 Astrocyte and Neuronal Assays

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The
10 selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF)
15 on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two
20 responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

25

Fibroblast and endothelial cell assays

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human
30 lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000

cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE₂ assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 α for 24 hours. The supernatants are collected and assayed for PGE₂ by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 α for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP⁺) and released. Subsequently, MPP⁺ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP⁺ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate:

ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989).

5 Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to
10 determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor
15 plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm² on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for
20 dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are
25 proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the
30 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells

5 On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at $2-5 \times 10^4$ cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and
10 positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

 An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

15 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

20 **Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells**

 For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was
25 performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF₁₆₅ or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to
30 wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is

subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al.* *In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to
5 test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell
Proliferation Stimulatory Effect**

10

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining
15 Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the
20 BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).

25

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 39: Stimulation of Endothelial Migration

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

5 Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate
10 concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber, 2.5×10^5 cells suspended in 50 ul M199 containing 1%
15 FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO₂ to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by
20 counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

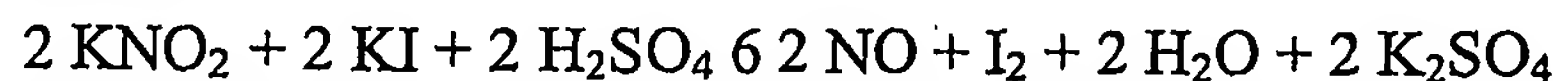
The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the
25 invention.

Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells

Nitric oxide released by the vascular endothelium is believed to be a mediator of
30 vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after
5 reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the
10 following equation:



The standard calibration curve is obtained by adding graded concentrations of KNO_2 (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and H_2SO_4 . The specificity of the Iso-NO electrode to NO is previously determined by
15 measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2
20 mm under the surface of the solution, before addition of the different conditions.

S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per 1×10^6 endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

25 The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 41: Effect of Polypeptides of the Invention on Cord Formation in Angiogenesis

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 µl/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 µg Cell Applications' Cord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. 17β-estradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old quail embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse

In vivo angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the

Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug
5 are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to
10 test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 44: Rescue of Ischemia in Rabbit Lower Limb Model

15 To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb
20 is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al.* *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid
25 containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al.* *Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al.* *J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1
30 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow

during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

Example 45: Effect of Polypeptides of the Invention on Vasodilation

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as $p < 0.05$ vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 46: Rat Ischemic Skin Flap Model

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

Ischemic skin

Ischemic skin wounds

Normal wounds

The experimental protocol includes:

5 Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

10 Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the
15 invention.

Example 47: Peripheral Arterial Disease Model

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic
20 strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

A polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered
25 intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

The studies described in this example tested activity of a polypeptide of the
30 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 48: Ischemic Myocardial Disease Model

5 A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

10 A polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

15 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 49: Rat Corneal Wound Healing Model

20

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

Making a 1-1.5 mm long incision from the center of cornea into the stromal layer. Inserting a spatula below the lip of the incision facing the outer corner of the eye. Making
25 a pocket (its base is 1-1.5 mm from the edge of the eye). Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.

Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

30 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models

5 *Diabetic db+/db+ Mouse Model.*

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is
10 dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal
15 heterozygous (db+/-m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375
20 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*,
25 *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of*
30 *Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/-m) heterozygous littermates are used in this study (Jackson Laboratories). The

animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional
5 Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01
10 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The
15 wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily
20 measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups
25 received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

30 Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm², the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)).
5 Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *Am. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating
10 monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl,
15 "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness
20 excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by
25 the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of
30 Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50

mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm^2 , the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus

microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a
5 blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to
10 test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 51: Lymphadema Animal Model

15 The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and
20 histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed
25 with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of
30 dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are

used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca^{2+} comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control
5 legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at -80°C until sectioning. Upon
10 sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

15

Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules
20 (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium
25 determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF- α), a potent proinflammatory cytokine, is a
30 stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF- α induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- α treated ECs when co-stimulated with a member of the FGF family of proteins.

5 To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO₂. HUVECs are seeded in 96-well plates at concentrations of 1×10^4 cells/well in EGM
10 medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

15 Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 μ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are
20 aspirated to remove medium and 100 μ l of 0.1% paraformaldehyde-PBS(with Ca⁺⁺ and Mg⁺⁺) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 μ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-
25 1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 μ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with
30 PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 μ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the

ExtrAvidin-Alkaline Phosphotase in glycine buffer: $1:5,000$ (10^0) $> 10^{-0.5} > 10^{-1} > 10^{-1.5}$. 5 μ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μ l of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 μ l of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

10 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

15 **Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation**

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

20 It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an

30

inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells
5 are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5×10^5 cells/ml. During this time, 100 μ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that
10 can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 μ l of prepared cytokines, 50 μ l SID (supernatants at 1:2 dilution = 50 μ l) and 20 μ l of diluted cells are added to the media which is already present in the wells to
15 allow for a final total volume of 100 μ l. The plates are then placed in a 37°C/5% CO₂ incubator for five days.

Eighteen hours before the assay is harvested, 0.5 μ Ci/well of [3H] Thymidine is added in a 10 μ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat
20 using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 μ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined
25 via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene
30 therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected

to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECCR)

The objective of the Extracellular Matrix Enhanced Cell Response (EMECCR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of $0.2 \mu\text{g}/\text{cm}^2$. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem

cells is to be expected. Gene products are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO₂, 7% O₂, and 88% N₂) tissue
5 culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA.

Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

10 One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular gene product is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene may be
15 useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

20 Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses
25 of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia
30 or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone

marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell

5 Proliferation

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF α stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 μ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 μ g/ml hEGF, 5mg/ml insulin, 1 μ g/ml hFGF, 50mg/ml gentamycin, 50 μ g/ml Amphotericin B, 5%FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50 μ g/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNF α is added to a final concentration of 2ng/ml

(NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO₂ until day 5.

Transfer 60µl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100 µl in
5 the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100
10 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash
15 buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled
20 Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

Add 100 µl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

25 A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product of interest. For example, inflammation and immune responses, wound healing, and
30 angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood

vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1

(VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μ l of 0.1% paraformaldehyde-PBS(with Ca⁺⁺ and Mg⁺⁺) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 μ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 μ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μ l of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 μ l of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-

conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 57: Alamar Blue Endothelial Cells Proliferation Assay

5 This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue
10 Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and
15 Angiostatin or TSP-1 are included as a known inhibitory controls.

 Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of
20 interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The
25 plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

 Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical
30 reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and

inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM[®], density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2×10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2×10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μ g/ml; anti-CD4 mAb (R&D

Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO₂, and 1 µC of [³H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Additionally, the content of U.S. provisional application Serial No. 60/234,211 is hereby incorporated by reference in its entirety.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description in Table 1 on page 58.

B. IDENTIFICATION OF DEPOSITFurther deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit
09 June 2000

Accession Number
PTA-2074

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*)This information is continued on an additional sheet ☐**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

	For receiving Office use only			For International Bureau use only	
<input type="checkbox"/> This sheet was received with the international application			<input type="checkbox"/> This sheet was received by the International Bureau on:		
Authorized officer			Authorized officer		

ATCC Deposit No. PTA-2074**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: 2074

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group
5 consisting of:

(a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID
10 NO:Y or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y
15 or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID
20 NO:X, having biological activity;

(f) a polynucleotide which is a variant of SEQ ID NO:X;

(g) a polynucleotide which is an allelic variant of SEQ ID NO:X;

(h) a polynucleotide which encodes a species homologue of the SEQ
ID NO:Y;

(i) a polynucleotide capable of hybridizing under stringent conditions
25 to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

30 2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence
5 included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to
10 SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
15

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

20 7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.
25

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

30 11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;

5 (c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(e) a secreted form of SEQ ID NO:Y or the encoded sequence
10 included in ATCC Deposit No:Z;

(f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(g) a variant of SEQ ID NO:Y;

(h) an allelic variant of SEQ ID NO:Y; or

15 (i) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

20 13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

25

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

30

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

5 18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

10 (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

15 (a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20 20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

25

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

30 (a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

<110> Human Genome Sciences, Inc.

<120> 21 Human Secreted Proteins

<130> PS725PCT

<140> Unassigned

<141> 2001-01-09

<150> 60/234,211

<151> 2000-09-20

<160> 139

<170> PatentIn Ver. 2.0

<210> 1

<211> 733

<212> DNA

<213> Homo sapiens

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acaagagcag	gtggcagcag	gggaacgtct	tctcatgctc	cgtgatgcat	gaggctctgc	660
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<210> 2

<211> 5

<212> PRT

<213> Homo sapiens

<220>

<221> Site

<222> (3)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 2

Trp	Ser	Xaa	Trp	Ser
1				5

<210> 3

<211> 86

<212> DNA

<213> Artificial Sequence

<220>

<221> Primer_Bind
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 <212> DNA
 <213> Artificial Sequence
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 <211> 271
 <212> DNA
 <213> Artificial Sequence
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 gccctaact ccgcccagtt ccgcccattc tccgccccat ggctgactaa ttttttttat 180
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<210> 6
 <211> 32
 <212> DNA
 <213> Artificial Sequence
 <220>
 <221> Primer_Bind
 <223> Synthetic primer complementary to human genomic EGR-1 promoter sequence (Sakamoto et al., Oncogene 6:867-871 (1991)); includes a Xho I restriction site.

<400> 6
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<210> 7
 <211> 31
 <212> DNA

<213> Artificial Sequence

<220>

<221> Primer_Bind

<223> Synthetic primer complementary to human genomic EGR-1 promoter sequence (Sakamoto et al., Oncogene 6:867-871 (1991)); includes a Hind III restriction site.

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31

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<400> 8

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12

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<220>

<221> Primer_Bind

<223> Synthetic primer with 4 tandem copies of the NF-KB binding site (GGGGACTTTCCC), 18 nucleotides complementary to the 5' end of the SV40 early promoter sequence, and a XhoI restriction site.

<400> 9

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ccatctcaat tag

60

73

<210> 10

<211> 256

<212> DNA

<213> Artificial Sequence

<220>

<221> Protein_Bind

<223> Synthetic promoter for use in biological assays; includes NF-KB binding sites.

<400> 10

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cagttccgcc cattctccgc cccatggctg actaattttt tttatttatg cagaggccga 180
ggccgcctcg gcctctgagc tattccagaa gtagtgagga ggcttttttg gaggcctagg 240
cttttgcaaa aagctt 256

<210> 11

<211> 2472

<212> DNA

<213> Homo sapiens

<400> 11

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<211> 4037

<212> DNA

<213> Homo sapiens

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<221> SITE

<222> (643)

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<210> 13

<211> 1593

<212> DNA

<213> Homo sapiens

<400> 13

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<212> DNA

<213> Homo sapiens

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<211> 2837

<212> DNA

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<210> 21
 <211> 1508
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (80)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (1498)
 <223> n equals a,t,g, or c

<400> 21
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 gccatggctg tctacgtcgg gatgctgcgc ctggggaggc tgtgcgccgg gagctcgggg 180
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<210> 22
 <211> 785
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (704)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (726)
 <223> n equals a,t,g, or c

<220>
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 <223> n equals a,t,g, or c

<220>
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 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (782)
 <223> n equals a,t,g, or c

<400> 22

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anttt						785

<210> 23
 <211> 514
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (508)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (509)
 <223> n equals a,t,g, or c

<400> 23

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atccacttag	gataaaagcc	tctagctgca	tccatgttac	tgcaaaggac	acgatttctt	240
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<210> 24

<211> 2286

<212> DNA

<213> Homo sapiens

<400> 24

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<210> 25

<211> 479

<212> DNA

<213> Homo sapiens

<400> 25

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<210> 26

<211> 644

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (529)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (561)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (582)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (593)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (615)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (621)

<223> n equals a,t,g, or c

<400> 26

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taaaaatccc	agcanctgga	ncaaggggta	gaggggctgg	gata		644

<210> 27
 <211> 1803
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (5)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (9)
 <223> n equals a,t,g, or c

<400> 27
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<210> 28
 <211> 1928
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE

<222> (695)

<223> n equals a,t,g, or c

<400> 28

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ctaaatca						1928

<210> 29

<211> 2045

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (2)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (5)

<223> n equals a,t,g, or c

<400> 29

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<210> 30

<211> 2089

<212> DNA

<213> Homo sapiens

<400> 30

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<210> 31
 <211> 807
 <212> DNA
 <213> Homo sapiens

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 <222> (128)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (634)
 <223> n equals a,t,g, or c

<400> 31						
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<210> 32
 <211> 433
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE

<222> (317)

<223> n equals a,t,g, or c

<400> 32

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<210> 33

<211> 1009

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (865)

<223> n equals a,t,g, or c

<400> 33

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<210> 34

<211> 683

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (5)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (534)

<223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (555)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (643)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (649)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (682)
 <223> n equals a,t,g, or c

<400> 34

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<210> 35
 <211> 3085
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (6)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (13)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (2137)
 <223> n equals a,t,g, or c

<400> 35

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<210> 36

<211> 773

<212> DNA

<213> Homo sapiens

25

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 <223> n equals a,t,g, or c

<220>
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 <222> (671)
 <223> n equals a,t,g, or c

<220>
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 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (722)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (731)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (755)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (764)
 <223> n equals a,t,g, or c

<400> 36

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 <212> DNA
 <213> Homo sapiens

<400> 37
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<210> 38

<211> 1837

<212> DNA

<213> Homo sapiens

<220>

<221> SITE
 <222> (417)
 <223> n equals a,t,g, or c

<220>
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 <222> (1813)
 <223> n equals a,t,g, or c

<220>
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 <222> (1827)
 <223> n equals a,t,g, or c

<400> 38

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<210> 39
 <211> 604
 <212> DNA
 <213> Homo sapiens

<400> 39

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<210> 40

<211> 1313

<212> DNA

<213> Homo sapiens

<400> 40

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<210> 41

<211> 1352

<212> DNA

<213> Homo sapiens

<400> 41

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<210> 42

<211> 1683

<212> DNA

<213> Homo sapiens

<400> 42

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<210> 43

<211> 982

<212> DNA

<213> Homo sapiens

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<221> SITE

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<220>
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<220>
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<400> 43

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<210> 44
 <211> 530
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (450)
 <223> n equals a,t,g, or c

<220>
 <221> SITE

<222> (496)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (511)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (517)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (519)
 <223> n equals a,t,g, or c

<400> 44

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attctcatta	ttatttat	at	tttttttt	agatgggtgc	tcgctctgtc	180
gagtgcagt	gcgtgacctt	agctcactgc	aaccaccacc	tactcgggag	gctgaggcag	240
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ctagcctgcg	taacgagcaa	aactctgtca	cacacacaca	caaaataata	acaataataa	360
taataaaaca	ttgccgggca	cagtggctca	tgccctgtaat	cccagcactt	tgggaggctg	420
aagctgggtg	atcgcggaag	caggagttcn	agaccagcct	ggccaagggtg	gtgaaacccc	480
atctctacta	aaaatnccaa	aattagccca	ncgtgngna	cacgccttgt		530

<210> 45
 <211> 541
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (420)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (528)
 <223> n equals a,t,g, or c

<400> 45

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tcctgcccta	cacacaccca	caggtagcgc	tccatctgct	attctgtgcc	ttgctttttt	180
cagacgcggt	aggaaaggcc	acatcagtca	tgacatacac	aggcttcttt	actcacagca	240
ctcattgtcg	ctttcatatt	tcttgcttta	gtctgagttt	tttaattcta	taagtaaaaa	300
catttttttaa	atccagaatt	caaaagtgat	cataaaagtt	attcctcctt	ttatcatata	360
ctctcagccg	tttatgtgga	gtaggagtg	gagtgtgtag	ccttccagac	ccttctgtgn	420
tacagaacag	aggaatttct	tattgaaatt	taactttgaa	ttgktatact	cgtaattttc	480
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t						541

<210> 46
 <211> 2183
 <212> DNA
 <213> Homo sapiens

<400> 46
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 tgccaattat tacttaaatg tgctcattct agcactgctt atgagttacg aaattaaaat 240
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 aagatgcctc atttgatata tttgaatgtt acatgttaca ttgtaaacad caagtaacac 720
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<210> 47
 <211> 2837
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (2771)
 <223> n equals a,t,g, or c

<400> 47
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tccttggatg	tctccatgat	aataattatt	tccttaggag	caatttgtgc	agtgttgctg	180
gttattatgg	tgctatttgc	aactaggtgt	aaccgcgaga	agaaagacac	tagatcctat	240
aactgcaggg	tggccgaatc	aacttaccag	caccacccaa	aaaggccatc	ccggcagatt	300
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cacagatcgt	ctccatcttc	atctcctacc	ttagaaagag	ggcagatggg	cagccggcag	420
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gagaatttct	cattagaact	cacccacgcc	actcctgctg	ttgaggtctc	tcagcttctt	540
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<210> 48

<211> 2718

<212> DNA

<213> Homo sapiens

<400> 48

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cgagaagaaa	gacactagat	cctataactg	caggggtggcc	gaatcaactt	accagcacca	180
cccaaaaagg	ccatcccggc	agattcacaa	aggggacatc	acattgggtgc	ctaccataaa	240

tggcactctg	cccatcagat	ctcatcacag	atcgtctcca	tcttcatctc	ctaccttaga	300
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<210> 49

<211> 571

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (472)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (508)

<223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (532)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (537)
 <223> n equals a,t,g, or c

<400> 49

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cttcaccttc	ctctgtgact	gtgaggcctc	cccagccatg	tgaaactgtg	agtccattaa	180
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<210> 50
 <211> 821
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (715)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (718)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (749)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (776)
 <223> n equals a,t,g, or c

<220>
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 <222> (788)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (821)
 <223> n equals a,t,g, or c

<400> 50

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tatgtgtaaa	tcctacaata	atgttcacct	aaacaaccac	aatgcaacca	taaaaatata	180
aacactttac	tacaatttat	ttatgtttgtg	ccatttgtcc	caaatatcct	ttgtcacaaa	240
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tctgttgccc	aggctgaagt	gcagtggcat	gatcttggct	cactacaatc	tccanctncg	720
gggtccaagc	gggtctcctg	cctcagctnc	aaagtaaagt	agctgagatt	acaggnacat	780
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<210> 51

<211> 625

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (606)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (612)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (625)

<223> n equals a,t,g, or c

<400> 51

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<210> 52

<211> 2074

<212> DNA

<213> Homo sapiens

<220>

<221> SITE
 <222> (18)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (200)
 <223> n equals a,t,g, or c

<400> 52

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<210> 53
 <211> 306
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (256)
 <223> n equals a,t,g, or c

<220>

<221> SITE
 <222> (301)
 <223> n equals a,t,g, or c

<400> 53
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 tctcttcagg tggagcctgc agttggcaaa gaacagctca gggagaggca gggccagag 120
 ctccctggct ggggtggctgg cctggccttt gtgtgtctct ttgcctgtgt tggggtgggg 180
 gtcgctccct gtcactcctt cgattctgaa gctgcctcct tctcctcct gtactcctgg 240
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 ntccct 306

<210> 54
 <211> 278
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (251)
 <223> n equals a,t,g, or c

<400> 54
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 atatatacca caggatatta agaacaagat catgtcctct gaagtaacat ggctggagct 120
 ggaggccatg accctaaggg aactgacaca ggcaagaaaa ccaaacatgg ctctcactta 180
 tgagtgggag ccaaacgctg agcacacatg gacacaaaga aggwwyaaga agtcagcagg 240
 gcctgggttg nggtggaggg agtgaggagg agggagag 278

<210> 55
 <211> 547
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (462)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (506)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (533)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (536)
 <223> n equals a,t,g, or c

<220>
 <221> SITE

<222> (545)

<223> n equals a,t,g, or c

<400> 55

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attgccgcca	gtgcacacgt	gaacctcgcc	actgctgccc	tgccccctgcc	agtgcagggtg	180
tacagtacat	gtgtgcatat	ggatgccacc	accccacatt	tgctgggtgtg	tacacaccct	240
cccacaccac	tgttgccacc	agcatatgca	cacagacacc	accacatcaa	tgctgctgga	300
gtgagcacac	gcatgtggtc	tctaccaccc	cactactgcc	ggcatacatg	cacatgagca	360
tggaccctgc	tgccaccacc	caaataaagt	gcttttgccg	gcaccccatc	agagcatttt	420
taccagtgga	gtgggaacac	ctcagcccct	tcaacacagc	angtgcttaa	cctcgagggg	480
gggcccggta	cccaattcgc	ctatantgag	tcgtattaca	attcactggc	cgncgnttta	540
caacntt						547

<210> 56

<211> 387

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (360)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (363)

<223> n equals a,t,g, or c

<400> 56

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acaggcgccc	gccaccacgc	ccggctaatt	ttttgtattt	ttagtagaga	cggggtttca	180
ccttgtttagc	caggatggtc	tcgatctctt	tacctcatga	tccacccgcc	tcggcctccc	240
aaagtgcagg	gattacaggc	gtgagcaccc	cgcccggccc	aacctgactt	acttgttcag	300
agtatttgct	agtagctttt	gtctgtagta	tttgtataaa	tattaatttt	tcatacataan	360
tgnttttagtt	tgataattta	tatatatt				387

<210> 57

<211> 1668

<212> DNA

<213> Homo sapiens

<400> 57

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cccttccatg	gtccccatct	tccttctgaa	atgtctactc	cttcatgttc	ctttatgtat	180
gtcttccaat	ctttccttcc	atagctctca	tcaccttcat	atatttcttc	catctttctc	240
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ctccccccat	atctttcctc	tatgtccaca	tctgtgtatt	ccccccaact	tcccctccat	360
atatcttttt	tactcccctt	ttcctccctg	tatcctctgt	gttcccccca	tcttgctcta	420
catcattctt	cccaagatct	ttacgtctcc	catcttgatc	tctccatctc	cactttctcc	480
taacattttc	atttccggtc	cttagtgtct	ctagagagat	cattcttgat	agcctcagct	540
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<210> 58

<211> 277

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (255)

<223> n equals a,t,g, or c

<400> 58

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tggcccccat	cttcccttctg	aaatgtctac	tccttcatgt	tcctttatgt	atgtcttcca	180
atctttcctt	ccatagctct	catcaccttc	atatatttct	tccatcttct	tcctcccacc	240
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<210> 59

<211> 3714

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (20)

<223> n equals a,t,g, or c

<400> 59

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<210> 60

<211> 1039

<212> DNA

<213> Homo sapiens

<220>
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 <222> (937)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
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 <223> n equals a,t,g, or c

<220>
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 <222> (971)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (994)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (997)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (1026)
 <223> n equals a,t,g, or c

<400> 60
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 ctaccaagaa cagctggggc tgggtggccac gtactttttg gggatcctga aagccaaggg 180
 gaccctgcga ccacctgagc gccaggccct gtttggtctc tgggagctca tctacggcgc 240
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 ccgccctgag tttggggggc ttcagctcca ggacctgctc cctctgcctc tgcaacggct 480
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<210> 61
 <211> 678
 <212> DNA
 <213> Homo sapiens

<220>

<221> SITE
 <222> (621)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (623)
 <223> n equals a,t,g, or c

<400> 61

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gatctggagt	agcttggtcc	aggcaaacag	aaagtgcctt	ttgtcaaatac	atgaagggtt	480
ctgtttttgt	tcagtactga	agattccttt	gtactcttgg	ctgtgactat	ccctgaggta	540
tcctgagttc	tggaatctat	aagattcctc	tagttttctg	gctgctgata	gccaagtca	600
gactgtggta	accagcgtga	nanctcctcc	tgggtctgtg	acataagcag	tagcttctca	660
cgagggaaag	gacagact					678

<210> 62
 <211> 1406
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (16)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (31)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (41)
 <223> n equals a,t,g, or c

<400> 62

gaaatactgt	aaaaancgcc	tgaaaatgac	ntaaacaagt	natgaatgta	aacacctggc	60
caaacaagcc	arctgtggac	ctgctcarcg	acctgaggcc	aagtgcgaga	aggtgtctga	120
gtttgtggcg	tctaagccag	gcacgtgtgt	gaaggtgctg	accatcgagc	ccccacctgc	180
agacccccgc	ctccgggagg	acatggcgct	gctggccgat	tgtgccctgc	cccccgagct	240
ccgaggtgat	ctttggggagc	tgcccttccc	ttgtcctgac	ggcttcaaca	gctgccctga	300
catctgcttc	cgagtggctg	gctgcagctt	cctctgccac	aaggcctttt	tctgtggccg	360
cagtgactac	ttccgagccc	tgctggatga	ccacttccga	gagagcgagg	agccagcgac	420
ctcagggggc	ccccagccg	tcaccctgca	tggcatctca	cccagcgtct	tcactcacgt	480
gctctactac	atgtacagcg	accacactga	gctgtccccc	gaggcagcct	atgatgtgct	540
gagcgtcgcc	gacatgtacc	tgctgccagg	cctgaagagg	ctgtgcggcc	gcagcctggc	600
tcagatgcta	gacgaggaca	ctgtgggtgg	tgtgtggcgc	gtggccaagc	tcttccgcct	660
ggcgcggctt	gaggaccagt	gcactgagta	catggccaag	gtcatttgaga	agctgggtga	720
gcgggaggac	ttcgtggagg	cggtgaagga	ggaggcagcg	gctgtggcag	cccggcagga	780

44

gacggactct	atcccgctgg	tggacgacat	cgccttccac	gtggccagca	cggtgcagac	840
ctacagcgcc	atagaggagg	cgcagcagcg	tctgcgggca	ctcgaggacc	tgctcgtgtc	900
catcggctctg	gactgttgag	cccctggctg	ggcagcccca	ggggccagga	gctctcttgg	960
agacaagcat	gtgtatgcgt	ttgtgtgcag	ctcttcttcc	tgctccctgc	acattgaggg	1020
cttcatgggg	ggtgcgaggg	gctcagtggg	gcttctcttc	cctccatgag	cctggagacc	1080
ccaggggagg	atccatttgg	gatgagcccc	ctccccccaa	tgcacaagcc	agcccccaag	1140
accctggggg	tggacaccac	tcagggaaac	ctgggggtggg	ggtgggcttt	ggtcttagca	1200
ctttccttct	ccagatcccc	cctaccacc	ccagtcccaa	atccagtcct	ctggcccttg	1260
cctagccctg	aattgcttct	ctaagctggt	gttcccatgc	acagggccat	tcaggaaggg	1320
ctgggggagt	gtgtgtggca	ataaagcttg	aaggcmccgt	grraaaaaaa	aaaaaaaaaa	1380
aaaaaaaaaa	aaaaaaaaaa	aaaaaa				1406

<210> 63
 <211> 771
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (751)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (758)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (767)
 <223> n equals a,t,g, or c

<400> 63						
gcggccgccc	tttttttttt	tttttttttaa	aaaactttttt	taatattttta	aacgagcatc	60
agaaccaggc	atggtatagt	cattcttcat	gaatgattct	cttggttttg	catcccttat	120
cagttgtcat	tgctccttaca	tagccttccct	ccccaaacag	attcttttta	atttattttc	180
cttagttcct	aaaatgagta	aaacttactt	tacaatacat	gtgggattat	gtgcagagat	240
tggttcctag	tgatttttgta	tagtattttat	cccttagcaa	atgtagttta	agaacatggt	300
aatctgatth	ccaatcacat	gatcagggtg	gtggatagtg	tcagtgccat	atgcaatggt	360
catgtcccag	gcatcaatga	tgcccacggt	gaggctcttg	aaaatatacct	tcatgataag	420
atagtgaata	taaccatgga	agtctccaaa	cctctctgtc	tctatgtgca	tctccctgat	480
gttttctgtc	ttaataatca	cttttagtggc	tgggcttctt	aggaacagtc	tttcaatagc	540
cttttgaaca	ccgatggccc	tgccaataaa	aatgtcaatg	ggaaatgggc	taaagtgtctg	600
gccaaagggtg	atgacgatgg	ctgtgttttt	gtcacctgat	agccgggtcaa	tttcccagag	660
gatataatca	tgatytatca	gagagtagrg	ctggaaagtg	acgaagggat	agctatgttt	720
tttccattga	attctgagtg	tgtctttctg	natccagnaa	gcaaaanggt	t	771

<210> 64
 <211> 1177
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (1172)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (1176)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (1177)
 <223> n equals a,t,g, or c

<400> 64

ctctaatac	g	astcactata	g	gggaaagctg	g	gtacgcctgc	a	aggtaccggt	c	ccggaattcc	60
cgggtcgacc	c	cacgcgtccg	a	agaactcaca	g	ggtacttttc	c	cagccacca	g	gataggagag	120
atcattaata	c	cagtgcattc	t	tgtgctacct	g	gacacctatt	g	gggacattcg	t	catgtcctc	180
aaatacaatg	c	ttcaaaaaa	c	gctgctgat	c	ttgatctct	t	ttttcagtag	t	aacctggat	240
gatttttata	a	ttttctcaga	a	acttcacaaa	g	gctttgggtc	t	gctctaaact	t	atccatctc	300
tgtccattac	t	ggaacaact	c	cgcgaagtc	c	ttattccct	a	aaacatcac	t	gataccatt	360
aaagccacta	a	cagagactg	a	aactcagaat	a	aaaggaaatc	a	atagagaaac	t	agatcagca	420
gatcccaccc	a	gacctttca	c	cccatgtgaa	c	caccaccacc	a	gtgccacac	a	cagcacagc	480
caccatcctc	a	accctcgag	a	atacatactg	c	caggggagac	c	cagctggaca	t	cctactgga	540
ggtgagggac	c	acttgggac	a	gaggaagca	a	atatgggtggg	g	gatttcctga	g	ggccaggat	600
gtcctcccca	g	cactgacgg	c	caggtgcttc	a	aggaaagggtg	a	atggacttca	a	caatggcac	660
ctacctgggc	a	gcttctactc	t	gtttctggga	g	ggccaggkc	t	tycctgkctc	t	gctgctyat	720
ycaccccagt	g	aagggggcgt	c	ggctytctg	g	aggggcaagg	a	aaccaaggct	a	tgataaaat	780
tattttcaaa	g	gcaaatttg	t	ttaatggcac	c	ctctcatgtc	t	ttactgaat	g	tggcctgac	840
cctaaactca	a	atgctgaac	t	tctgtgaata	t	tctggatgac	a	gagaccaag	a	agccttcta	900
ttgtatgaag	c	ctcaacaca	t	gccctgtga	g	gctctgacc	t	acatgacca	c	ccggaatag	960
agaggtatct	t	atcttacag	a	caaggaaaa	c	cagccttttc	c	cacaggtcca	a	agtgggagt	1020
tgaaatgatg	a	aggatcgta	a	acacattga	t	gkcactaat	t	gkaacaaga	g	agaaaamat	1080
atgaagagac	a	tgccaaagt	g	gaatgaagc	c	tyctgtccc	t	tggtggttat	a	ctttacaag	1140
gaaaatggat	a	acmacattt	t	ggaaaccag	g	nttann					1177

<210> 65
 <211> 684
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (648)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (651)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (678)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (679)
 <223> n equals a,t,g, or c

<220>

<221> SITE

<222> (682)

<223> n equals a,t,g, or c

<400> 65

gtcgacccac	gcgtccgaga	actcacaggt	acttttccag	ccacccagat	aggagagatc	60
attaaacag	tgcattctgt	gctacctgac	acctattggg	acattcgtca	tgtcctcaaa	120
tacaatgctt	caaaaaacgc	tgctgatctt	gatctctttt	tcagtagtaa	cctggatgat	180
ttttataatt	tctcagaact	tcacaaagct	ttgggtctgct	ctaaacttat	ccatctctgt	240
ccattactgg	aacaactccg	caaagtcctt	attccctaaa	acatcactga	taccattaaa	300
gccactaaca	gagactgaac	tcagaataaa	ggaaatcata	gagaaactag	atcagcagat	360
cccacccaga	cctttcaccc	atgtgaacac	caccaccagt	gccacacaca	gcacagccac	420
catcctcaac	cctcgagata	catactgcag	gggagaccag	ctggacatcc	tactggaggt	480
gagggaccac	ttgggacaga	ggaagcaata	tggtggggat	ttcctgaggg	ccaggatgtc	540
cttcccagca	ctgacggcag	gtgcttcagg	aaaggtgatg	gacttcacaa	tggcacctac	600
ctggcagctt	cactctggtc	tgggagggcc	agggttctct	ggctctgntg	nttattcacc	660
ccaagtggaa	ggggccgnng	gntt				684

<210> 66

<211> 771

<212> DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (751)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (758)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (767)

<223> n equals a,t,g, or c

<400> 66

gcggccgccc	tttttttttt	ttttttttta	aaaacttttt	taatatttta	aacgagcatc	60
agaaccaggc	atgggtatagt	cattcttcat	gaatgattct	cttggttttg	catcccttat	120
cagttgtcat	tgtccttaca	tagccttcct	cccaaacag	attcttttaa	atttattttc	180
cttagttcct	aaaatgagta	aaacttactt	tacaatacat	gtgggattat	gtgcagagat	240
tggttcctag	tgattttgta	tagtatttat	cccttagcaa	atgtagttta	agaacatggt	300
aatctgattt	ccaatcacat	gatcagggtg	gtggatagtg	tcagtgccat	atgcaatggg	360
catgtcccag	gcatcaatga	tgcccacggt	gaggtctttg	aaaatatcct	tcatgataag	420
atagtgaata	taaccatgga	agtctccaaa	cctctctgtc	tctatgtgca	tctccctgat	480
gttttctgtc	ttaataatca	ctttagtggc	tgggcttctt	aggaacagtc	tttcaatagc	540
cttttgaaca	ccgatggccc	tgccaataaa	aatgtcaatg	ggaaatggtc	taaagtgctg	600
gccaaagggtg	atgacgatgg	ctgtgttttt	gtcacctgat	agccgggtcaa	tttcccagg	660
gatataatca	tgatytatca	gagagtagrg	ctggaaagtg	acgaagggat	agctatgttt	720
tttccattga	attctgagtg	tgtctttctg	natccagnaa	gcaaaanggt	t	771

<210> 67

<211> 822
 <212> DNA
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (649)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (751)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (770)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (807)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (814)
 <223> n equals a,t,g, or c

<220>
 <221> SITE
 <222> (820)
 <223> n equals a,t,g, or c

<400> 67
 tcgaggtcga cggtatcgat aagcttgata tcgaattccc aaggagcagc cccctcccct 60
 gcagcccccc cagcagtcga tcagagtga ggaggagcag tacctcgggc acgaggggtcc 120
 aggaggggca gtctccacct ctcagcctgt ggaactgccc cctcctagca gcctggccct 180
 gctgaactct gtggtatatg ggcctgagcg gacctcagca gccatgctgt cccagcaggt 240
 ggcctcagta aagtggccca actctgtgat ggctccaggc cggggcccgg agcgtggagg 300
 aggtgggggt gtcagtga ca gca gctggca gcagcagcca rgccagcctc caccaccattc 360
 aacatggaac tgccacagtc tgtccctcta cagtgcmaacc aaggggagcc cgcatacctgg 420
 agtgggagtc ccgacttact ataaccaccc tgaggcactg aagmgggaga aagcgggggg 480
 cccacagctg gaccgctatg tgcgaccaat gatgccacag aaggtgcagc tggaggtagg 540
 gcggccccag gcaccctga attctttcca cgcagccaag aaacccccaa accagtcact 600
 gccctgcaa cccttccagc tggcattcgg scaccargtg aaccggcang tcttccggca 660
 gggcccaccg cccccaacc cggtggctgc cttccctcca cagaagcagc agcagcagca 720
 gcaaccacag cagcagcagc agcagcagca nggaagccct aaccagatn gccgctcttt 780
 tgagaacttc tatttccatg ccgcagnaac cctnggaagn aa 822

<210> 68
 <211> 128
 <212> PRT
 <213> Homo sapiens

<400> 68
 Met Val Val Ala Val Leu Leu Gly Phe Val Ala Met Val Leu Ser Val

48

1	5	10	15
Val Gly Met Lys Cys Thr Arg Val Gly Asp Ser Asn Pro Ile Ala Lys	20	25	30
Gly Arg Val Ala Ile Ala Gly Gly Ala Leu Phe Ile Leu Ala Gly Leu	35	40	45
Cys Thr Leu Thr Ala Val Ser Trp Tyr Ala Thr Leu Val Thr Gln Glu	50	55	60
Phe Phe Asn Pro Ser Thr Pro Val Asn Ala Arg Tyr Glu Phe Gly Pro	65	70	75
Ala Leu Phe Val Gly Trp Ala Ser Ala Gly Leu Ala Val Leu Gly Gly	85	90	95
Ser Phe Leu Cys Cys Thr Cys Pro Glu Pro Glu Arg Pro Asn Ser Ser	100	105	110
Pro Gln Pro Tyr Arg Pro Gly Pro Ser Ala Ala Ala Arg Glu Tyr Val	115	120	125

<210> 69

<211> 320

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (168)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 69

Met Asn Leu Ser Thr Ala Leu Leu Phe Leu Asn Leu Leu Phe Leu Leu	1	5	10	15
Asp Gly Trp Ile Thr Ser Phe Asn Val Asp Gly Leu Cys Ile Ala Val	20	25	30	
Ala Val Leu Leu His Phe Phe Leu Leu Ala Thr Phe Thr Trp Met Gly	35	40	45	
Leu Glu Ala Ile His Met Tyr Ile Ala Leu Val Lys Val Phe Asn Thr	50	55	60	
Tyr Ile Arg Arg Tyr Ile Leu Lys Phe Cys Ile Ile Gly Trp Gly Leu	65	70	75	80
Pro Ala Leu Val Val Ser Val Val Leu Ala Ser Arg Asn Asn Asn Glu	85	90	95	
Val Tyr Gly Lys Glu Ser Tyr Gly Lys Glu Lys Gly Asp Glu Phe Cys	100	105	110	

Trp Ile Gln Asp Pro Val Ile Phe Tyr Val Thr Cys Ala Gly Tyr Phe
 115 120 125
 Gly Val Met Phe Phe Leu Asn Ile Ala Met Phe Ile Val Val Met Val
 130 135 140
 Gln Ile Cys Gly Arg Asn Gly Lys Arg Ser Asn Arg Thr Leu Arg Glu
 145 150 155 160
 Glu Val Leu Arg Asn Leu Arg Xaa Val Val Ser Leu Thr Phe Leu Leu
 165 170 175
 Gly Met Thr Trp Gly Phe Ala Phe Phe Ala Trp Gly Pro Leu Asn Ile
 180 185 190
 Pro Phe Met Tyr Leu Phe Ser Ile Phe Asn Ser Leu Gln Gly Leu Phe
 195 200 205
 Ile Phe Ile Phe His Cys Ala Met Lys Glu Asn Val Gln Lys Gln Trp
 210 215 220
 Arg Arg His Leu Cys Cys Gly Arg Phe Arg Leu Ala Asp Asn Ser Asp
 225 230 235 240
 Trp Ser Lys Thr Ala Thr Asn Ile Ile Lys Lys Ser Ser Asp Asn Leu
 245 250 255
 Gly Lys Ser Leu Ser Ser Ser Ser Ile Gly Ser Asn Ser Thr Tyr Leu
 260 265 270
 Thr Ser Lys Ser Lys Ser Ser Ser Thr Thr Tyr Phe Lys Arg Asn Ser
 275 280 285
 His Thr Asp Asn Val Ser Tyr Glu His Ser Phe Asn Lys Ser Gly Ser
 290 295 300
 Leu Arg Gln Cys Phe His Gly Gln Val Leu Val Lys Thr Gly Pro Cys
 305 310 315 320

<210> 70
 <211> 473
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (321)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (386)
 <223> Xaa equals any of the naturally occurring L-amino acids

50

<220>

<221> SITE

<222> (391)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 70

Met	Ala	Ser	Ala	Val	Arg	Gly	Ser	Arg	Pro	Trp	Pro	Arg	Leu	Gly	Leu
1				5					10				15		

Gln	Leu	Gln	Phe	Ala	Ala	Leu	Leu	Leu	Gly	Thr	Leu	Ser	Pro	Gln	Val
			20					25					30		

His	Thr	Leu	Arg	Pro	Glu	Asn	Leu	Leu	Leu	Val	Ser	Thr	Leu	Asp	Gly
		35					40					45			

Ser	Leu	His	Ala	Leu	Ser	Lys	Gln	Thr	Gly	Asp	Leu	Lys	Trp	Thr	Leu
	50					55					60				

Arg	Asp	Asp	Pro	Val	Ile	Glu	Gly	Pro	Met	Tyr	Val	Thr	Glu	Met	Ala
65					70					75					80

Phe	Leu	Ser	Asp	Pro	Ala	Asp	Gly	Ser	Leu	Tyr	Ile	Leu	Gly	Thr	Gln
				85					90					95	

Lys	Gln	Gln	Gly	Leu	Met	Lys	Leu	Pro	Phe	Thr	Ile	Pro	Glu	Leu	Val
			100					105					110		

His	Ala	Ser	Pro	Cys	Arg	Ser	Ser	Asp	Gly	Val	Phe	Tyr	Thr	Gly	Arg
		115					120					125			

Lys	Gln	Asp	Ala	Trp	Phe	Val	Val	Asp	Pro	Glu	Ser	Gly	Glu	Thr	Gln
	130					135					140				

Met	Thr	Leu	Thr	Thr	Glu	Gly	Pro	Ser	Thr	Pro	Arg	Leu	Tyr	Ile	Gly
145					150					155					160

Arg	Thr	Gln	Tyr	Thr	Val	Thr	Met	His	Asp	Pro	Arg	Ala	Pro	Ala	Leu
				165					170					175	

Arg	Trp	Asn	Thr	Thr	Tyr	Arg	Arg	Tyr	Ser	Ala	Pro	Pro	Met	Asp	Gly
		180						185					190		

Ser	Pro	Gly	Lys	Tyr	Met	Ser	His	Leu	Ala	Ser	Cys	Gly	Met	Gly	Leu
		195					200					205			

Leu	Leu	Thr	Val	Asp	Pro	Gly	Ser	Gly	Thr	Val	Leu	Trp	Thr	Gln	Asp
	210					215					220				

Leu	Gly	Val	Pro	Val	Met	Gly	Val	Tyr	Thr	Trp	His	Gln	Asp	Gly	Leu
225					230					235					240

Arg	Gln	Leu	Pro	His	Leu	Thr	Leu	Ala	Arg	Asp	Thr	Leu	His	Phe	Leu
				245					250					255	

Ala	Leu	Arg	Trp	Gly	His	Ile	Arg	Leu	Pro	Ala	Ser	Gly	Pro	Arg	Asp
		260						265					270		

51

Thr Ala Thr Leu Phe Ser Thr Leu Asp Thr Gln Leu Leu Met Thr Leu
 275 280 285
 Tyr Val Gly Lys Asp Glu Thr Gly Phe Tyr Val Ser Lys Ala Leu Val
 290 295 300
 His Thr Gly Val Ala Leu Val Pro Arg Gly Leu Thr Leu Ala Pro Ala
 305 310 315 320
 Xaa Gly Pro Thr Thr Asp Glu Val Thr Leu Gln Val Ser Gly Glu Arg
 325 330 335
 Glu Gly Ser Pro Ser Thr Ala Val Arg Tyr Pro Ser Gly Ser Val Ala
 340 345 350
 Leu Pro Ser Gln Trp Leu Leu Ile Gly His His Glu Leu Pro Pro Val
 355 360 365
 Leu His Thr Thr Met Leu Arg Val His Pro Thr Leu Gly Ser Gly Thr
 370 375 380
 Ala Xaa Thr Arg Pro Pro Xaa Asn Thr Gln Ala Pro Ala Phe Phe Leu
 385 390 395 400
 Glu Leu Leu Ser Leu Ser Arg Glu Lys Leu Trp Asp Ser Glu Leu His
 405 410 415
 Pro Glu Glu Lys Thr Pro Asp Ser Tyr Leu Gly Leu Gly Pro Gln Asp
 420 425 430
 Leu Leu Ala Ala Ser Leu Thr Ala Val Leu Leu Gly Gly Trp Ile Leu
 435 440 445
 Phe Val Met Arg Gln Gln Gln Pro Gln Val Val Glu Lys Gln Gln Glu
 450 455 460
 Thr Pro Leu Ala Pro Ala Ala Trp Gly
 465 470

<210> 71
 <211> 497
 <212> PRT
 <213> Homo sapiens

<400> 71
 Met Phe Leu Asp Arg Pro Gln Gln Trp Leu Gln Leu Val Leu Leu Pro
 1 5 10 15
 Pro Ala Leu Phe Ile Pro Ser Thr Glu Asn Glu Glu Gln Arg Leu Ala
 20 25 30
 Ser Ala Arg Ala Val Pro Arg Asn Val Gln Pro Tyr Val Val Tyr Glu
 35 40 45
 Glu Val Thr Asn Val Trp Ile Asn Val His Asp Ile Phe Tyr Pro Phe
 50 55 60

52

Pro Gln Ser Glu Gly Glu Asp Glu Leu Cys Phe Leu Arg Ala Asn Glu
 65 70 75 80
 Cys Lys Thr Gly Phe Cys His Leu Tyr Lys Val Thr Ala Val Leu Lys
 85 90 95
 Ser Gln Gly Tyr Asp Trp Ser Glu Pro Phe Ser Pro Gly Glu Asp Glu
 100 105 110
 Phe Lys Cys Pro Ile Lys Glu Glu Ile Ala Leu Thr Ser Gly Glu Trp
 115 120 125
 Glu Val Leu Ala Arg His Gly Ser Lys Ile Trp Val Asn Glu Glu Thr
 130 135 140
 Lys Leu Val Tyr Phe Gln Gly Thr Lys Asp Thr Pro Leu Glu His His
 145 150 155 160
 Leu Tyr Val Val Ser Tyr Glu Ala Ala Gly Glu Ile Val Arg Leu Thr
 165 170 175
 Thr Pro Gly Phe Ser His Ser Cys Ser Met Ser Gln Asn Phe Asp Met
 180 185 190
 Phe Val Ser His Tyr Ser Ser Val Ser Thr Pro Pro Cys Val His Val
 195 200 205
 Tyr Lys Leu Ser Gly Pro Asp Asp Asp Pro Leu His Lys Gln Pro Arg
 210 215 220
 Phe Trp Ala Ser Met Met Glu Ala Ala Ser Cys Pro Pro Asp Tyr Val
 225 230 235 240
 Pro Pro Glu Ile Phe His Phe His Thr Arg Ser Asp Val Arg Leu Tyr
 245 250 255
 Gly Met Ile Tyr Lys Pro His Ala Leu Gln Pro Gly Lys Lys His Pro
 260 265 270
 Thr Val Leu Phe Val Tyr Gly Gly Pro Gln Val Gln Leu Val Asn Asn
 275 280 285
 Ser Phe Lys Gly Ile Lys Tyr Leu Arg Leu Asn Thr Leu Ala Ser Leu
 290 295 300
 Gly Tyr Ala Val Val Val Ile Asp Gly Arg Gly Ser Cys Gln Arg Gly
 305 310 315 320
 Leu Arg Phe Glu Gly Ala Leu Lys Asn Gln Met Gly Gln Val Glu Ile
 325 330 335
 Glu Asp Gln Val Glu Gly Leu Gln Phe Val Ala Glu Lys Tyr Gly Phe
 340 345 350
 Ile Asp Leu Ser Arg Val Ala Ile His Gly Trp Ser Tyr Gly Gly Phe
 355 360 365
 Leu Ser Leu Met Gly Leu Ile His Lys Pro Gln Val Phe Lys Val Ala

53

370 375 380
 Ile Ala Gly Ala Pro Val Thr Val Trp Met Ala Tyr Asp Thr Gly Tyr
 385 390 395 400
 Thr Glu Arg Tyr Met Asp Val Pro Glu Asn Asn Gln His Gly Tyr Glu
 405 410 415
 Ala Gly Ser Val Ala Leu His Val Glu Lys Leu Pro Asn Glu Pro Asn
 420 425 430
 Arg Leu Leu Ile Leu His Gly Phe Leu Asp Glu Asn Val His Phe Phe
 435 440 445
 His Thr Asn Phe Leu Val Ser Gln Leu Ile Arg Ala Gly Lys Pro Tyr
 450 455 460
 Gln Leu Gln Ile Tyr Pro Asn Glu Arg His Ser Ile Arg Cys Pro Glu
 465 470 475 480
 Ser Gly Glu His Tyr Glu Val Thr Leu Leu His Phe Leu Gln Glu Tyr
 485 490 495

Leu

<210> 72
 <211> 255
 <212> PRT
 <213> Homo sapiens

<400> 72
 Met Lys Val Leu Ala Thr Ser Phe Val Leu Gly Ser Leu Gly Leu Ala
 1 5 10 15
 Phe Tyr Leu Pro Leu Val Val Thr Thr Pro Lys Thr Leu Ala Ile Pro
 20 25 30
 Glu Lys Leu Gln Glu Ala Val Gly Lys Val Ile Ile Asn Ala Thr Thr
 35 40 45
 Cys Thr Val Thr Cys Gly Leu Gly Tyr Lys Glu Glu Thr Val Cys Glu
 50 55 60
 Val Gly Pro Asp Gly Val Arg Arg Lys Cys Gln Thr Arg Arg Leu Glu
 65 70 75 80
 Cys Leu Thr Asn Trp Ile Cys Gly Met Leu His Phe Thr Ile Leu Ile
 85 90 95
 Gly Lys Glu Phe Glu Leu Ser Cys Leu Ser Ser Asp Ile Leu Glu Phe
 100 105 110
 Gly Gln Glu Ala Phe Arg Phe Thr Trp Arg Leu Ala Arg Gly Val Ile
 115 120 125
 Ser Thr Asp Asp Glu Val Phe Lys Pro Phe Gln Ala Asn Ser His Phe

54

130	135	140
Val Lys Phe Lys Tyr Ala Gln Glu Tyr Asp Ser Gly Thr Tyr Arg Cys		
145	150	155 160
Asp Val Gln Leu Val Lys Asn Leu Arg Leu Val Lys Arg Leu Tyr Phe		
	165	170 175
Gly Leu Arg Val Leu Pro Pro Asn Leu Val Asn Leu Asn Phe His Gln		
	180	185 190
Ser Leu Thr Glu Asp Gln Lys Leu Ile Asp Glu Gly Leu Glu Val Asn		
	195	200 205
Leu Asp Ser Tyr Ser Lys Pro His His Pro Lys Trp Lys Lys Lys Val		
	210	215 220
Ala Ser Ala Leu Gly Ile Gly Ile Ala Ile Gly Val Val Gly Gly Val		
	225	230 235 240
Leu Val Arg Ile Val Leu Cys Ala Leu Arg Gly Gly Leu Gln Gln		
	245	250 255

<210> 73
 <211> 87
 <212> PRT
 <213> Homo sapiens

<400> 73
 Met Thr Gly Gln Ile Pro Arg Leu Ser Lys Val Asn Leu Phe Thr Leu
 1 5 10 15
 Leu Ser Leu Trp Met Glu Leu Phe Pro Ala Glu Ala Gln Arg Gln Lys
 20 25 30
 Ser Gln Lys Asn Glu Glu Gly Lys His Gly Pro Leu Gly Asp Asn Glu
 35 40 45
 Glu Arg Thr Arg Val Ser Thr Asp Lys Arg Gln Lys Thr Met Phe Cys
 50 55 60
 Leu Phe Glu Asn Asp Cys Lys Cys Lys Ala Leu Arg Val Met Ile Arg
 65 70 75 80
 Ser Met Ser Arg Ser Val Pro
 85

<210> 74
 <211> 100
 <212> PRT
 <213> Homo sapiens

<400> 74
 Met Val Arg Thr Arg Ala Leu Phe Tyr Ile Phe Phe Gln Leu Ser Leu
 1 5 10 15

55

Thr Ser Gly Leu Ile Glu Asp Ser Cys Ile Leu Ile Ile Ile Tyr Leu
 20 25 30
 Phe Phe Phe Arg Trp Cys Leu Ala Leu Ser Pro Met Leu Glu Cys Ser
 35 40 45
 Gly Val Thr Leu Ala His Cys Asn His His Leu Leu Gly Arg Leu Arg
 50 55 60
 Gln Glu Asn Arg Leu Asn Leu Gly Gly Gly Asp Cys Ser Glu Leu Arg
 65 70 75 80
 Leu His His Cys Thr Leu Ala Cys Val Thr Ser Lys Thr Leu Ser His
 85 90 95
 Thr His Thr Lys
 100

<210> 75
 <211> 94
 <212> PRT
 <213> Homo sapiens

<400> 75
 Met Pro His Ser Ser Leu Tyr Pro Pro Pro Phe Phe Lys Met Lys Leu
 1 5 10 15
 Ile Ile Arg Val Trp Phe Ile Ile Ser Leu Phe Phe Val Gln Gly Arg
 20 25 30
 Thr Asn Pro Cys Ile Leu Leu Pro Tyr Thr His Pro Gln Val Ala Leu
 35 40 45
 His Leu Leu Phe Cys Ala Leu Leu Phe Ser Asp Ala Leu Gly Lys Ala
 50 55 60
 Thr Ser Val Met Thr Tyr Thr Gly Phe Phe Thr His Ser Thr His Cys
 65 70 75 80
 Arg Phe His Ile Ser Cys Phe Ser Leu Ser Phe Leu Ile Leu
 85 90

<210> 76
 <211> 447
 <212> PRT
 <213> Homo sapiens

<400> 76
 Met Thr Ser Val Ser Gln Ala Ser Leu Asp Val Ser Met Ile Ile Ile
 1 5 10 15
 Ile Ser Leu Gly Ala Ile Cys Ala Val Leu Leu Val Ile Met Val Leu
 20 25 30
 Phe Ala Thr Arg Cys Asn Arg Glu Lys Lys Asp Thr Arg Ser Tyr Asn
 35 40 45

56

Cys	Arg	Val	Ala	Glu	Ser	Thr	Tyr	Gln	His	His	Pro	Lys	Arg	Pro	Ser
50						55					60				
Arg	Gln	Ile	His	Lys	Gly	Asp	Ile	Thr	Leu	Val	Pro	Thr	Ile	Asn	Gly
65					70					75					80
Thr	Leu	Pro	Ile	Arg	Ser	His	His	Arg	Ser	Ser	Pro	Ser	Ser	Ser	Pro
				85					90					95	
Thr	Leu	Glu	Arg	Gly	Gln	Met	Gly	Ser	Arg	Gln	Ser	His	Asn	Ser	His
			100					105					110		
Gln	Ser	Leu	Asn	Ser	Leu	Val	Thr	Ile	Ser	Ser	Asn	His	Val	Pro	Glu
		115					120					125			
Asn	Phe	Ser	Leu	Glu	Leu	Thr	His	Ala	Thr	Pro	Ala	Val	Glu	Val	Ser
	130					135					140				
Gln	Leu	Leu	Ser	Met	Leu	His	Gln	Gly	Gln	Tyr	Gln	Pro	Arg	Pro	Ser
145					150					155					160
Phe	Arg	Gly	Asn	Lys	Tyr	Ser	Arg	Ser	Tyr	Arg	Tyr	Ala	Leu	Gln	Asp
				165					170					175	
Met	Asp	Lys	Phe	Ser	Leu	Lys	Asp	Ser	Gly	Arg	Gly	Asp	Ser	Glu	Ala
		180						185					190		
Gly	Asp	Ser	Asp	Tyr	Asp	Leu	Gly	Arg	Asp	Ser	Pro	Ile	Asp	Arg	Leu
		195					200					205			
Leu	Gly	Glu	Gly	Phe	Ser	Asp	Leu	Phe	Leu	Thr	Asp	Gly	Arg	Ile	Pro
	210					215					220				
Ala	Ala	Met	Arg	Leu	Cys	Thr	Glu	Glu	Cys	Arg	Val	Leu	Gly	His	Ser
225					230					235					240
Asp	Gln	Cys	Trp	Met	Pro	Pro	Leu	Pro	Ser	Pro	Ser	Ser	Asp	Tyr	Arg
				245					250					255	
Ser	Asn	Met	Phe	Ile	Pro	Gly	Glu	Glu	Phe	Pro	Thr	Gln	Pro	Gln	Gln
		260						265					270		
Gln	His	Pro	His	Gln	Ser	Leu	Glu	Asp	Asp	Ala	Gln	Pro	Ala	Asp	Ser
		275					280					285			
Gly	Glu	Lys	Lys	Lys	Ser	Phe	Ser	Thr	Phe	Gly	Lys	Asp	Ser	Pro	Asn
	290					295					300				
Asp	Glu	Asp	Thr	Gly	Asp	Thr	Ser	Thr	Ser	Ser	Leu	Leu	Ser	Glu	Met
305					310					315					320
Ser	Ser	Val	Phe	Gln	Arg	Leu	Leu	Pro	Pro	Ser	Leu	Asp	Thr	Tyr	Ser
				325					330					335	
Glu	Cys	Ser	Glu	Val	Asp	Arg	Ser	Asn	Ser	Leu	Glu	Arg	Arg	Lys	Gly
			340					345					350		

57

Pro Leu Pro Ala Lys Thr Val Gly Tyr Pro Gln Gly Val Ala Ala Trp
 355 360 365

Ala Ala Ser Thr His Phe Gln Asn Pro Thr Thr Asn Cys Gly Pro Pro
 370 375 380

Leu Gly Thr His Ser Ser Val Gln Pro Ser Ser Lys Trp Leu Pro Ala
 385 390 395 400

Met Glu Glu Ile Pro Glu Asn Tyr Glu Glu Asp Asp Phe Asp Asn Val
 405 410 415

Leu Asn His Leu Asn Asp Gly Lys His Glu Leu Met Asp Ala Ser Glu
 420 425 430

Leu Val Ala Glu Ile Asn Lys Leu Leu Gln Asp Val Arg Gln Ser
 435 440 445

<210> 77
 <211> 93
 <212> PRT
 <213> Homo sapiens

<400> 77
 Met Ile Val Asn Ile Ser His Glu Ile Trp Trp Phe Tyr Lys Gly Lys
 1 5 10 15

Val Pro Leu His Met Leu Thr Cys Leu Leu Pro Cys Lys Thr Cys Leu
 20 25 30

Ala Pro Pro Ser Pro Ser Ser Val Thr Val Arg Pro Pro Gln Pro Cys
 35 40 45

Glu Thr Val Ser Pro Leu Lys Leu Phe Phe Phe Ile Asn Tyr Pro Val
 50 55 60

Leu His Met Ser Leu Leu Thr Val Arg Lys Trp Thr Asn Thr Leu Gly
 65 70 75 80

His Glu Gly Gly Ala Leu Ile Asn Gly Ile Ser Ala Leu
 85 90

<210> 78
 <211> 461
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (424)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (459)
 <223> Xaa equals any of the naturally occurring L-amino acids

58

<400> 78

Met	Ala	Val	Tyr	Val	Gly	Met	Leu	Arg	Leu	Gly	Arg	Leu	Cys	Ala	Gly	1	5	10	15
Ser	Ser	Gly	Val	Leu	Gly	Ala	Arg	Ala	Ala	Leu	Ser	Arg	Ser	Trp	Gln	20	25	30	
Glu	Ala	Arg	Leu	Gln	Gly	Val	Arg	Phe	Leu	Ser	Ser	Arg	Glu	Val	Asp	35	40	45	
Arg	Met	Val	Ser	Thr	Pro	Ile	Gly	Gly	Leu	Ser	Tyr	Val	Gln	Gly	Cys	50	55	60	
Thr	Lys	Lys	His	Leu	Asn	Ser	Lys	Thr	Val	Gly	Gln	Cys	Leu	Glu	Thr	65	70	75	80
Thr	Ala	Gln	Arg	Val	Pro	Glu	Arg	Glu	Ala	Leu	Val	Val	Leu	His	Glu	85	90	95	
Asp	Val	Arg	Leu	Thr	Phe	Ala	Gln	Leu	Lys	Glu	Glu	Val	Asp	Lys	Ala	100	105	110	
Ala	Ser	Gly	Leu	Leu	Ser	Ile	Gly	Leu	Cys	Lys	Gly	Asp	Arg	Leu	Gly	115	120	125	
Met	Trp	Gly	Pro	Asn	Ser	Tyr	Ala	Trp	Val	Leu	Met	Gln	Leu	Ala	Thr	130	135	140	
Ala	Gln	Ala	Gly	Ile	Ile	Leu	Val	Ser	Val	Asn	Pro	Ala	Tyr	Gln	Ala	145	150	155	160
Met	Glu	Leu	Glu	Tyr	Val	Leu	Lys	Lys	Val	Gly	Cys	Lys	Ala	Leu	Val	165	170	175	
Phe	Pro	Lys	Gln	Phe	Lys	Thr	Gln	Gln	Tyr	Tyr	Asn	Val	Leu	Lys	Gln	180	185	190	
Ile	Cys	Pro	Glu	Val	Glu	Asn	Ala	Gln	Pro	Gly	Ala	Leu	Lys	Ser	Gln	195	200	205	
Arg	Leu	Pro	Asp	Leu	Thr	Thr	Val	Ile	Ser	Val	Asp	Ala	Pro	Leu	Pro	210	215	220	
Gly	Thr	Leu	Leu	Leu	Asp	Glu	Val	Val	Ala	Ala	Gly	Ser	Thr	Arg	Gln	225	230	235	240
His	Leu	Asp	Gln	Leu	Gln	Tyr	Asn	Gln	Gln	Phe	Leu	Ser	Cys	His	Asp	245	250	255	
Pro	Ile	Asn	Ile	Gln	Phe	Thr	Ser	Gly	Thr	Thr	Gly	Ser	Pro	Lys	Gly	260	265	270	
Ala	Thr	Leu	Ser	His	Tyr	Asn	Ile	Val	Asn	Asn	Ser	Asn	Ile	Leu	Gly	275	280	285	
Glu	Arg	Leu	Lys	Leu	His	Glu	Lys	Thr	Pro	Glu	Gln	Leu	Arg	Met	Ile	290	295	300	

59

Leu Pro Asn Pro Leu Tyr His Cys Leu Gly Ser Val Ala Gly Thr Met
 305 310 315 320
 Met Cys Leu Met Tyr Gly Ala Thr Leu Ile Leu Ala Ser Pro Ile Phe
 325 330 335
 Asn Gly Lys Lys Ala Leu Glu Ala Ile Ser Arg Glu Arg Gly Thr Phe
 340 345 350
 Leu Tyr Gly Thr Pro Thr Met Phe Val Asp Ile Leu Asn Gln Pro Asp
 355 360 365
 Phe Ser Ser Tyr Asp Ile Ser Thr Met Cys Gly Gly Val Ile Ala Gly
 370 375 380
 Ser Pro Ala Pro Pro Glu Leu Ile Arg Ala Ile Ile Asn Lys Ile Asn
 385 390 395 400
 Met Lys Asp Leu Val Val Ala Tyr Gly Thr Thr Glu Asn Ser Pro Val
 405 410 415
 Thr Phe Ala His Phe Pro Glu Xaa Thr Pro Lys Pro Leu Asp Lys Glu
 420 425 430
 Lys Arg Ala Glu Tyr Ala Ser His Gly Gly Glu Pro Leu Thr Lys Thr
 435 440 445
 Ser Lys Ser His Leu Pro Ser Pro Ser Trp Xaa Gly Ser
 450 455 460

<210> 79

<211> 113

<212> PRT

<213> Homo sapiens

<400> 79

Met Ser Pro Ser Pro Arg Trp Gly Phe Leu Cys Val Leu Phe Thr Ala
 1 5 10 15
 Val His Pro Ala Pro Ser Thr Ala Pro Val Gln Asp Lys Cys Pro Val
 20 25 30
 Asn Thr Trp Glu Ala Met Gln Ala Ser Ser Gln Gln Leu Leu Gln Thr
 35 40 45
 Asp Pro Arg Pro Lys Pro Phe Leu Leu Pro Pro Leu Pro Pro Leu Leu
 50 55 60
 Leu Ile Ser Ala Gly Thr Glu Val Ser Ser Leu Val Phe Gln Lys Ser
 65 70 75 80
 Pro Leu His Thr Gln Pro Glu Gly Ala Ile Lys Thr Ala Gly Gln Pro
 85 90 95
 Thr Ser Val His Ser Lys Val Leu Ser Lys Gly Ser Leu Leu Leu Gly
 100 105 110

Glu

<210> 80
 <211> 100
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (62)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (85)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (88)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (95)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (99)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 80
 Met Leu Leu Gln Arg Thr Arg Phe Leu Leu Leu Phe Phe Ser Phe Val
 1 5 10 15

Ser Ser Phe Phe Leu Ser Leu Pro Ser Phe Ser Leu Phe Phe Leu Phe
 20 25 30

Leu Ser Leu Ser Leu Phe Cys Ile His Val Ala Ala Lys Asp Met Ile
 35 40 45

Ser Ser Phe Phe Ser Leu Pro Phe Ser Phe Leu Ser Phe Xaa Leu Ser
 50 55 60

Phe Leu Leu Pro Ser Phe Ser Phe Phe Tyr Phe Phe Phe Phe Trp Leu
 65 70 75 80

Ser Phe Phe Phe Xaa Ser Lys Xaa Leu Ala Leu Val Pro Lys Xaa Gly
 85 90 95

Met Gln Xaa Val
 100

61

<210> 81
 <211> 168
 <212> PRT
 <213> Homo sapiens

<400> 81
 Met Val Val Met Ala Ser Leu Gln Val Glu Pro Ala Val Gly Lys Glu
 1 5 10 15
 Gln Leu Arg Glu Arg Gln Gly Pro Glu Leu Leu Gly Trp Val Ala Gly
 20 25 30
 Leu Ala Phe Val Cys Leu Phe Ala Cys Val Gly Val Gly Val Ala Pro
 35 40 45
 Cys His Ser Phe Asp Ser Glu Ala Ala Ser Phe Leu Leu Leu Tyr Ser
 50 55 60
 Trp Cys Thr Pro Arg Leu Leu Ser Trp Leu Arg Asp Thr Pro Ser Pro
 65 70 75 80
 Leu Ala Ser Gly Thr Phe Pro Pro His Ser Pro Leu Gly Glu Arg Pro
 85 90 95
 Leu Leu Ser Gly Pro Pro Ser Ser Ser Gln Gln Leu Leu Val Val Gly
 100 105 110
 Pro Cys Ala Leu Arg Phe Val Gly Ala Arg His Val Lys Thr Ala Gly
 115 120 125
 Phe Arg Asp Gly Phe Ser Leu Pro Ser Ser Ser Val Phe Ser Glu Phe
 130 135 140
 Trp Lys Met Thr Leu Leu Glu Ala Pro Leu Leu Cys His Leu Ser Ser
 145 150 155 160
 Lys Ser Gly Ala Ser Ala Cys Trp
 165

<210> 82
 <211> 141
 <212> PRT
 <213> Homo sapiens

<400> 82
 Met Gly Pro Arg Gly Cys Ala Leu Ala His Ser Leu Leu Pro Leu Leu
 1 5 10 15
 Cys Gln His Val Trp Thr Ser Pro Arg Tyr Cys Arg Gln Cys Thr Arg
 20 25 30
 Glu Pro Arg His Cys Cys Pro Ala Pro Ala Ser Ala Gly Val Gln Tyr
 35 40 45
 Met Cys Ala Tyr Gly Cys His His Pro Thr Phe Ala Gly Val Tyr Thr
 50 55 60

62

Pro Ser His Thr Thr Val Ala Thr Ser Ile Cys Thr Gln Thr Pro Pro
 65 70 75 80

His Gln Cys Cys Trp Ser Glu His Thr His Val Val Ser Thr Thr Pro
 85 90 95

Leu Leu Pro Ala Tyr Met His Met Ser Met Asp Pro Ala Ala Thr Thr
 100 105 110

Gln Met Lys Cys Phe Cys Arg His Pro Ile Arg Ala Phe Leu Pro Val
 115 120 125

Glu Trp Glu His Leu Ser Pro Ser Asn Thr Ala Gly Ala
 130 135 140

<210> 83
 <211> 94
 <212> PRT
 <213> Homo sapiens

<400> 83
 Met Gly Val Leu Trp Tyr Thr Phe Trp Tyr Thr Phe Thr Leu Leu Glu
 1 5 10 15

Cys Ser Arg Ser Ser Asn Asp Ser Arg Thr Leu Val Leu Ile Cys Leu
 20 25 30

Ser Leu Leu Gly Phe Asp Phe Val Arg Val Leu Asn Ile Lys Leu Ala
 35 40 45

Val Gly Glu Ser Thr Leu His Met Leu Ser Leu Pro Phe Ser Leu Arg
 50 55 60

Leu Ser Pro Ala Leu Pro Phe Ser Pro Phe Leu Leu Leu Met Asn Lys
 65 70 75 80

Pro Leu Ser Asp Val Gln Tyr Phe Asn Leu His Phe Ala Gly
 85 90

<210> 84
 <211> 125
 <212> PRT
 <213> Homo sapiens

<400> 84
 Met Val Pro Ile Phe Leu Leu Lys Cys Leu Leu Leu His Val Pro Leu
 1 5 10 15

Cys Met Ser Ser Asn Leu Ser Phe His Ser Ser His His Leu His Ile
 20 25 30

Phe Leu Pro Ser Phe Ser Ser His Leu Pro Arg Pro Leu Tyr Ile Pro
 35 40 45

Pro Leu Ser Pro Phe Tyr Ile Phe Ser Ile Ser Pro His Ile Phe Pro
 50 55 60

63

Leu Cys Pro His Leu Cys Ile Pro Pro Asn Phe Pro Ser Ile Tyr Leu
 65 70 75 80

Phe Tyr Ser Pro Phe Pro Pro Cys Ile Leu Cys Val Pro Pro Ile Leu
 85 90 95

Leu Tyr Ile Ile Leu Pro Lys Ile Phe Thr Ser Pro Ile Leu Ile Ser
 100 105 110

Pro Ser Pro Leu Ser Pro Asn Ile Phe Ile Ser Val Pro
 115 120 125

<210> 85
 <211> 211
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (21)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (104)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 85
 Met Ser Gly Met Thr Leu Ser Ser Thr Asp Met Tyr Thr Val Ser Leu
 1 5 10 15

Leu Leu Cys Leu Xaa Phe Lys Lys Ser Asp Pro Asp Pro Gly Pro Phe
 20 25 30

Gln Asn Asn Leu Phe His Asn His Gly Thr Gln Ser Gln Ser Cys Met
 35 40 45

Gly Ser Lys Val Gly Asp Val Ile Pro Gly Ala Ala Arg Leu Ile Ser
 50 55 60

Glu Thr Ala Gln Arg Val His Thr Ile Gly Gln Lys Gln Lys Asn Asp
 65 70 75 80

Gln His Leu Arg Arg Val Gln Ala Leu Leu Ser Gly Arg Gln Ala Lys
 85 90 95

Gly Leu Thr Ser Gly Arg Trp Xaa Leu Arg Gln Gly Trp Leu Leu Val
 100 105 110

Val Pro Pro His Gly Glu Pro Arg Pro Arg Met Phe Phe Leu Phe Thr
 115 120 125

Asp Val Leu Leu Met Ala Lys Pro Arg Pro Pro Leu His Leu Leu Arg
 130 135 140

Ser Gly Thr Phe Ala Cys Lys Ala Leu Tyr Pro Met Ala Gln Cys His

64

145 150 155 160
 Leu Ser Arg Val Phe Gly His Ser Gly Gly Pro Cys Gly Gly Leu Leu
 165 170 175
 Ser Leu Ser Phe Pro Arg Glu Lys Leu Leu Leu Met Ser Thr Asp Gln
 180 185 190
 Glu Glu Leu Ser Arg Trp Tyr His Ser Leu Thr Trp Ala Ile Ser Ser
 195 200 205
 Gln Lys Asn
 210

<210> 86
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 86
 Met Cys Met Arg Leu Cys Ala Ala Leu Leu Pro Ala Pro Cys Thr Leu
 1 5 10 15
 Arg Ala Ser Trp Gly Val Arg Gly Ala Gln Trp Gly Phe Ser Ser Leu
 20 25 30
 His Glu Pro Gly Asp Pro Arg Gly Gly Ser Ile Trp Asp Glu Pro Pro
 35 40 45
 Pro Pro Asn Ala Gln Ala Ser Pro Gln Asp Pro Gly Gly Gly His His
 50 55 60
 Ser Gly Lys Pro Gly Val Gly Val Gly Phe Gly Leu Ser Thr Phe Leu
 65 70 75 80
 Leu Gln Ile Pro Pro Thr His Pro Ser Pro Lys Ser Ser Pro Leu Ala
 85 90 95
 Leu Ala

<210> 87
 <211> 547
 <212> PRT
 <213> Homo sapiens

<400> 87
 Met Ser Ser Asn Thr Met Leu Gln Lys Thr Leu Leu Ile Leu Ile Ser
 1 5 10 15
 Phe Ser Val Val Thr Trp Met Ile Phe Ile Ile Ser Gln Asn Phe Thr
 20 25 30
 Lys Leu Trp Ser Ala Leu Asn Leu Ser Ile Ser Val His Tyr Trp Asn
 35 40 45

65

Asn	Ser	Ala	Lys	Ser	Leu	Phe	Pro	Lys	Thr	Ser	Leu	Ile	Pro	Leu	Lys
50						55					60				
Pro	Leu	Thr	Glu	Thr	Glu	Leu	Arg	Ile	Lys	Glu	Ile	Ile	Glu	Lys	Leu
65					70					75					80
Asp	Gln	Gln	Ile	Pro	Pro	Arg	Pro	Phe	Thr	His	Val	Asn	Thr	Thr	Thr
				85					90					95	
Ser	Ala	Thr	His	Ser	Thr	Ala	Thr	Ile	Leu	Asn	Pro	Arg	Asp	Thr	Tyr
			100					105					110		
Cys	Arg	Gly	Asp	Gln	Leu	Asp	Ile	Leu	Leu	Glu	Val	Arg	Asp	His	Leu
		115					120					125			
Gly	Gln	Arg	Lys	Gln	Tyr	Gly	Gly	Asp	Phe	Leu	Arg	Ala	Arg	Met	Ser
		130				135					140				
Ser	Pro	Ala	Leu	Thr	Ala	Gly	Ala	Ser	Gly	Lys	Val	Met	Asp	Phe	Asn
145					150					155					160
Asn	Gly	Thr	Tyr	Leu	Val	Ser	Phe	Thr	Leu	Phe	Trp	Glu	Gly	Gln	Val
				165					170					175	
Ser	Leu	Ser	Leu	Leu	Leu	Ile	His	Pro	Ser	Glu	Gly	Ala	Ser	Ala	Leu
			180					185						190	
Trp	Arg	Ala	Arg	Asn	Gln	Gly	Tyr	Asp	Lys	Ile	Ile	Phe	Lys	Gly	Lys
		195					200					205			
Phe	Val	Asn	Gly	Thr	Ser	His	Val	Phe	Thr	Glu	Cys	Gly	Leu	Thr	Leu
	210					215					220				
Asn	Ser	Asn	Ala	Glu	Leu	Cys	Glu	Tyr	Leu	Asp	Asp	Arg	Asp	Gln	Glu
225					230					235					240
Ala	Phe	Tyr	Cys	Met	Lys	Pro	Gln	His	Met	Pro	Cys	Glu	Ala	Leu	Thr
				245					250					255	
Tyr	Met	Thr	Thr	Arg	Asn	Arg	Glu	Val	Ser	Tyr	Leu	Thr	Asp	Lys	Glu
			260					265					270		
Asn	Ser	Leu	Phe	His	Arg	Ser	Lys	Val	Gly	Val	Glu	Met	Met	Lys	Asp
		275					280					285			
Arg	Lys	His	Ile	Asp	Val	Thr	Asn	Cys	Asn	Lys	Arg	Glu	Lys	Ile	Glu
	290					295					300				
Glu	Thr	Cys	Gln	Val	Gly	Met	Lys	Pro	Pro	Val	Pro	Gly	Gly	Tyr	Thr
305					310					315					320
Leu	Gln	Gly	Lys	Trp	Ile	Thr	Thr	Phe	Cys	Asn	Gln	Val	Gln	Leu	Asp
				325					330					335	
Thr	Ile	Lys	Ile	Asn	Gly	Cys	Leu	Lys	Gly	Lys	Leu	Ile	Tyr	Leu	Leu
			340					345					350		
Gly	Asp	Ser	Thr	Leu	Arg	Gln	Trp	Ile	Tyr	Tyr	Phe	Pro	Lys	Val	Val

66

355 360 365
 Lys Thr Leu Lys Phe Phe Asp Leu His Glu Thr Gly Ile Phe Lys Lys
 370 375 380
 His Leu Leu Leu Asp Ala Glu Arg His Thr Gln Ile Gln Trp Lys Lys
 385 390 395 400
 His Ser Tyr Pro Phe Val Thr Phe Gln Leu Tyr Ser Leu Ile Asp His
 405 410 415
 Asp Tyr Ile Pro Arg Glu Ile Asp Arg Leu Ser Gly Asp Lys Asn Thr
 420 425 430
 Ala Ile Val Ile Thr Phe Gly Gln His Phe Arg Pro Phe Pro Ile Asp
 435 440 445
 Ile Phe Ile Arg Arg Ala Ile Gly Val Gln Lys Ala Ile Glu Arg Leu
 450 455 460
 Phe Leu Arg Ser Pro Ala Thr Lys Val Ile Ile Lys Thr Glu Asn Ile
 465 470 475 480
 Arg Glu Met His Ile Glu Thr Glu Arg Phe Gly Asp Phe His Gly Tyr
 485 490 495
 Ile His Tyr Leu Ile Met Lys Asp Ile Phe Lys Asp Leu Asn Val Gly
 500 505 510
 Ile Ile Asp Ala Trp Asp Met Thr Ile Ala Tyr Gly Thr Asp Thr Ile
 515 520 525
 His Pro Pro Asp His Val Ile Gly Asn Gln Ile Asn Met Phe Leu Asn
 530 535 540
 Tyr Ile Cys
 545

<210> 88
 <211> 111
 <212> PRT
 <213> Homo sapiens

<400> 88
 Met Arg Met Ser Leu Ala Asp Ser Leu Ala Cys Ser Val Cys Val Ala
 1 5 10 15
 Leu Thr Ala Ala Ala Arg Leu Leu Arg Ser Arg Pro Ser Ser Cys Ser
 20 25 30
 Ser Phe Ser Trp Ile Ser Gly Thr Ser Ser Ser Pro Ser Phe Leu Gly
 35 40 45
 Ser Phe Thr Ser Leu Leu Gly Ser Ser Leu Ser Ser Leu Gly Asp Ser
 50 55 60
 Leu Leu Gly Arg Gly Thr Leu Gly Asn Phe Trp Glu Val Leu Ile Ser

67

65		70		75		80
Thr Ser Thr Ser Ser Trp Ala Asp Phe Ser Ser Leu Val Ser Thr Ser						
		85		90		95
Pro Lys Val Arg Val Pro Leu Arg Pro Ile Phe Thr Cys Phe Leu						
		100		105		110

<210> 89
 <211> 124
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (63)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (86)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 89
Met Val Val Ala Val Leu Leu Gly Phe Val Ala Met Val Leu Ser Val
1 5 10 15
Val Gly Met Lys Cys Thr Arg Val Gly Asp Ser Asn Pro Ile Ala Lys
20 25 30
Gly Arg Val Ala Ile Ala Gly Gly Ala Leu Phe Ile Leu Ala Gly Leu
35 40 45
Cys Thr Leu Thr Ala Val Ser Trp Tyr Ala Thr Leu Val Thr Xaa Glu
50 55 60
Phe Phe Asn Pro Ser Thr Pro Val Asn Ala Arg Tyr Glu Phe Gly Pro
65 70 75 80
Ala Leu Phe Val Gly Xaa Asp Ser Ala Gly Leu Ala Val Leu Ser Gly
85 90 95
Ser Phe Leu Cys Cys Thr Cys Pro Glu Pro Glu Arg Pro Asn Ser Ser
100 105 110
Pro Gln Ala Leu Ser Ala Trp Thr Leu Cys Cys Cys
115 120

<210> 90
 <211> 97
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (49)

68

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 90

Asn Ser Ala Arg Asp Gln Ala Ser Gly Glu Ser Ile His His Arg Thr
1 5 10 15

Ser Pro Ser Leu Pro Arg Thr Phe Leu Gly Gln Leu His Ser Gly Leu
20 25 30

Leu His His Leu Pro Cys Asp His Ile Ser His His Val Pro Arg Ser
35 40 45

Xaa Glu Arg Ser Ser Ala Ser Pro Ser Ser Leu Thr Leu Arg Gly Lys
50 55 60

Val Thr Glu Thr Lys Ser Asp Glu Met Thr Ala Met Tyr Thr Ala Val
65 70 75 80

Lys Gly Arg Glu Gly Arg Asn Asp Thr Asn Gly Arg Glu Leu Leu Gly
85 90 95

Asn

<210> 91

<211> 181

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (132)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (139)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (168)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (170)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (181)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 91

Met Asn Leu Ser Thr Ala Leu Leu Phe Leu Asn Leu Leu Phe Leu Leu
1 5 10 15

69

Asp Gly Trp Ile Thr Ser Phe Asn Val Asp Gly Leu Cys Ile Ala Val
 20 25 30
 Ala Val Leu Leu His Phe Phe Leu Leu Ala Thr Phe Thr Trp Met Gly
 35 40 45
 Leu Glu Ala Ile His Met Tyr Ile Ala Leu Val Lys Val Phe Asn Thr
 50 55 60
 Tyr Ile Arg Arg Tyr Ile Leu Lys Phe Cys Ile Ile Gly Trp Gly Leu
 65 70 75 80
 Pro Ala Leu Val Val Ser Val Val Leu Ala Ser Arg Asn Asn Asn Glu
 85 90 95
 Val Tyr Gly Lys Glu Ser Tyr Gly Lys Glu Lys Gly Asp Glu Phe Cys
 100 105 110
 Trp Ile Gln Asp Pro Val Ile Phe Tyr Val Thr Cys Ala Gly Tyr Phe
 115 120 125
 Gly Val Met Xaa Phe Leu Asn Ile Ala Met Xaa Ile Val Val Met Val
 130 135 140
 Gln Ile Cys Gly Arg Asn Gly Lys Arg Ser Asn Arg Thr Leu Arg Glu
 145 150 155 160
 Glu Val Val Arg Asn Leu Arg Xaa Val Xaa Ser Leu Thr Phe Leu Val
 165 170 175
 Gly Met Thr Trp Xaa
 180

<210> 92
 <211> 118
 <212> PRT
 <213> Homo sapiens

<400> 92
 Cys Arg Gly Asp Ile Gln Ile Arg Asp Lys Gly Glu Ala Met Leu Arg
 1 5 10 15
 Lys Thr Leu Asp Arg Ala His Phe Thr Pro Pro Asn Arg Tyr Ile Trp
 20 25 30
 Ile Tyr Pro Phe Ser Ala Ser Ser Phe Ser Thr Ile Lys Asn Val Thr
 35 40 45
 Ile Leu Asn Ala His Lys Ser His Ser Ser Val Thr Phe Cys Glu Cys
 50 55 60
 Ser Thr Ile Phe Ser Phe Ser Met Thr Phe Gln Pro Gln Ala Glu Lys
 65 70 75 80
 Thr Val Tyr Ser Leu Thr Gln Arg Leu Lys Arg Ile Phe Tyr Tyr Phe
 85 90 95

70

Lys Tyr Tyr Thr Phe Arg Thr Ile Thr Cys Leu Arg Lys Leu Ser Gln
 100 105 110

Asn Val Asp Leu Val Lys
 115

<210> 93
 <211> 246
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (30)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (212)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (220)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (243)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 93
 Met Ala Ser Ala Val Arg Gly Ser Arg Pro Trp Pro Arg Leu Gly Leu
 1 5 10 15

Gln Leu Gln Phe Ala Ala Leu Leu Leu Gly Thr Leu Ser Xaa Gln Val
 20 25 30

His Thr Leu Arg Pro Glu Asn Leu Leu Leu Val Ser Thr Leu Asp Gly
 35 40 45

Ser Leu His Ala Leu Ser Lys Gln Thr Gly Asp Leu Lys Trp Thr Leu
 50 55 60

Arg Asp Asp Pro Val Ile Glu Gly Pro Met Tyr Val Thr Glu Met Ala
 65 70 75 80

Phe Leu Ser Asp Pro Ala Asp Gly Ser Leu Tyr Ile Leu Gly Thr Gln
 85 90 95

Lys Gln Gln Gly Leu Met Lys Leu Pro Phe Thr Ile Pro Glu Leu Val
 100 105 110

His Ala Ser Pro Cys Arg Ser Ser Asp Gly Val Phe Tyr Thr Gly Arg
 115 120 125

71

Lys Gln Asp Ala Trp Phe Val Val Asp Pro Glu Ser Gly Glu Thr Gln
 130 135 140
 Met Thr Leu Thr Thr Glu Gly Pro Ser Thr Pro Arg Leu Tyr Ile Gly
 145 150 155 160
 Arg Thr Gln Tyr Thr Val Thr Met His Asp Pro Arg Ala Pro Ala Leu
 165 170 175
 Arg Trp Asn Thr Thr Tyr Arg Arg Tyr Ser Thr Pro Pro Met Asp Gly
 180 185 190
 Ser Thr Gly Lys Tyr Met Ser Gln Leu Gly Val Leu Arg Glu Gly Pro
 195 200 205
 Ala Ala His Xaa Gly Thr Pro Gly Ser Gly Thr Xaa Leu Leu Asp Thr
 210 215 220
 Arg Asn Leu Gly Arg Ala Leu Gly Asn Gly Pro Ala Thr Pro Leu Gly
 225 230 235 240
 Thr Lys Xaa Arg Ala Trp
 245

<210> 94

<211> 497

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (183)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 94

Met Phe Leu Asp Arg Pro Gln Gln Trp Leu Gln Leu Val Leu Leu Pro
 1 5 10 15

Pro Ala Leu Phe Ile Pro Ser Thr Glu Asn Glu Glu Gln Arg Leu Ala
 20 25 30

Ser Ala Arg Ala Val Pro Arg Asn Val Gln Pro Tyr Val Val Tyr Glu
 35 40 45

Glu Val Thr Asn Val Trp Ile Asn Val His Asp Ile Phe Tyr Pro Phe
 50 55 60

Pro Gln Ser Glu Gly Glu Asp Glu Leu Cys Phe Leu Arg Ala Asn Glu
 65 70 75 80

Cys Lys Thr Gly Phe Cys His Leu Tyr Lys Val Thr Ala Val Leu Lys
 85 90 95

Ser Gln Gly Tyr Asp Trp Ser Glu Pro Phe Ser Pro Gly Glu Asp Glu
 100 105 110

Phe Lys Cys Pro Ile Lys Glu Glu Ile Ala Leu Thr Ser Gly Glu Trp

115					120					125					
Glu	Val	Leu	Ala	Arg	His	Gly	Ser	Lys	Ile	Trp	Val	Asn	Glu	Glu	Thr
130						135					140				
Lys	Leu	Val	Tyr	Phe	Gln	Gly	Thr	Lys	Asp	Thr	Pro	Leu	Glu	His	His
145					150					155					160
Leu	Tyr	Val	Val	Ser	Tyr	Glu	Ala	Ala	Gly	Glu	Ile	Val	Arg	Leu	Thr
				165					170					175	
Thr	Pro	Gly	Phe	Ser	His	Xaa	Cys	Ser	Met	Ser	Gln	Asn	Phe	Asp	Met
			180					185					190		
Phe	Val	Ser	His	Tyr	Ser	Ser	Val	Ser	Thr	Pro	Pro	Cys	Val	His	Val
		195					200					205			
Tyr	Lys	Leu	Ser	Gly	Pro	Asp	Asp	Asp	Pro	Leu	His	Lys	Gln	Pro	Arg
	210					215					220				
Phe	Trp	Ala	Ser	Met	Met	Glu	Ala	Ala	Ser	Cys	Pro	Pro	Asp	Tyr	Val
225					230					235					240
Pro	Pro	Glu	Ile	Phe	His	Phe	His	Thr	Arg	Ser	Asp	Val	Arg	Leu	Tyr
				245					250					255	
Gly	Met	Ile	Tyr	Lys	Pro	His	Ala	Leu	Gln	Pro	Gly	Lys	Lys	His	Pro
		260						265					270		
Thr	Val	Leu	Phe	Val	Tyr	Gly	Gly	Pro	Gln	Val	Gln	Leu	Val	Asn	Asn
		275					280					285			
Ser	Phe	Lys	Gly	Ile	Lys	Tyr	Leu	Arg	Leu	Asn	Thr	Leu	Ala	Ser	Leu
	290					295					300				
Gly	Tyr	Ala	Val	Val	Val	Ile	Asp	Gly	Arg	Gly	Ser	Cys	Gln	Arg	Gly
305					310					315					320
Leu	Arg	Phe	Glu	Gly	Ala	Leu	Lys	Asn	Gln	Met	Gly	Gln	Val	Glu	Ile
				325					330					335	
Glu	Asp	Gln	Val	Glu	Gly	Leu	Gln	Phe	Val	Ala	Glu	Lys	Tyr	Gly	Phe
			340					345					350		
Ile	Asp	Leu	Ser	Arg	Val	Ala	Ile	His	Gly	Trp	Ser	Tyr	Gly	Gly	Phe
		355					360					365			
Leu	Ser	Leu	Met	Gly	Leu	Ile	His	Lys	Pro	Gln	Val	Phe	Lys	Val	Ala
	370					375					380				
Ile	Ala	Gly	Ala	Pro	Val	Thr	Val	Trp	Met	Ala	Tyr	Asp	Thr	Gly	Tyr
385					390					395					400
Thr	Glu	Arg	Tyr	Met	Asp	Val	Pro	Glu	Asn	Asn	Gln	His	Gly	Tyr	Glu
				405					410					415	
Ala	Gly	Ser	Val	Ala	Leu	His	Val	Glu	Lys	Leu	Pro	Asn	Glu	Pro	Asn
			420					425					430		

73

Arg Leu Leu Ile Leu His Gly Phe Leu Asp Glu Asn Val His Phe Phe
 435 440 445

His Thr Asn Phe Leu Val Ser Gln Leu Ile Arg Ala Gly Lys Pro Tyr
 450 455 460

Gln Leu Gln Ile Tyr Pro Asn Glu Arg His Ser Ile Arg Cys Pro Glu
 465 470 475 480

Ser Gly Glu His Tyr Glu Val Thr Leu Leu His Phe Leu Gln Glu Tyr
 485 490 495

Leu

<210> 95
 <211> 349
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (183)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (191)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (192)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (334)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (344)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (345)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (348)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 95

74

Met	Phe	Leu	Asp	Arg	Pro	Gln	Gln	Trp	Leu	Gln	Leu	Val	Leu	Leu	Pro	1	5	10	15
Pro	Ala	Leu	Phe	Ile	Pro	Ser	Thr	Glu	Asn	Glu	Glu	Gln	Arg	Leu	Ala	20	25	30	
Ser	Ala	Arg	Ala	Val	Pro	Arg	Asn	Val	Gln	Pro	Tyr	Val	Val	Tyr	Glu	35	40	45	
Glu	Val	Thr	Asn	Val	Trp	Ile	Asn	Val	His	Asp	Ile	Phe	Tyr	Pro	Phe	50	55	60	
Pro	Gln	Ser	Glu	Gly	Glu	Asp	Glu	Leu	Cys	Phe	Leu	Arg	Ala	Asn	Glu	65	70	75	80
Cys	Lys	Thr	Gly	Phe	Cys	His	Leu	Tyr	Lys	Val	Thr	Ala	Val	Leu	Lys	85	90	95	
Ser	Gln	Gly	Tyr	Asp	Trp	Ser	Glu	Pro	Phe	Ser	Pro	Gly	Glu	Asp	Glu	100	105	110	
Phe	Lys	Cys	Pro	Ile	Lys	Glu	Glu	Ile	Ala	Leu	Thr	Ser	Gly	Glu	Trp	115	120	125	
Glu	Val	Leu	Ala	Arg	His	Gly	Ser	Lys	Ile	Trp	Val	Asn	Glu	Glu	Thr	130	135	140	
Lys	Leu	Val	Tyr	Phe	Gln	Gly	Thr	Lys	Asp	Thr	Pro	Leu	Glu	His	His	145	150	155	160
Leu	Tyr	Val	Val	Ser	Tyr	Glu	Ala	Ala	Gly	Glu	Ile	Val	Arg	Leu	Thr	165	170	175	
Thr	Pro	Gly	Phe	Ser	His	Xaa	Cys	Ser	Met	Ser	Gln	Asn	Phe	Xaa	Xaa	180	185	190	
Phe	Val	Ser	His	Ile	Thr	Ala	Gln	Val	Ala	Ala	Ala	Ser	Ala	Gly	Asn	195	200	205	
Gln	Ala	Gly	Gly	Thr	Glu	Trp	Pro	Ala	Gly	Pro	Ser	Glu	Ala	Leu	Cys	210	215	220	
Pro	Ala	Gln	Arg	Trp	Pro	Ala	Pro	Arg	Ser	Arg	Cys	Leu	His	Arg	Pro	225	230	235	240
Asp	Ala	Phe	Tyr	Pro	Phe	Leu	Asn	Ala	Leu	Gly	Phe	Tyr	Val	Arg	Cys	245	250	255	
Phe	Leu	Val	Ala	Glu	Thr	Glu	Arg	Trp	Trp	Ser	Arg	Ala	Ser	Pro	Ser	260	265	270	
Ser	Pro	Arg	Leu	Leu	Gly	Gly	Gly	Gly	His	Thr	Leu	Met	Gly	Thr	Gly	275	280	285	
Glu	Ala	Arg	Arg	Asp	Ser	Glu	Glu	Arg	Ala	Ala	Phe	Arg	Leu	Gly	Leu	290	295	300	
Pro	Val	Thr	Ser	Gln	Ser	Pro	Gly	Pro	Ala	Ser	His	Arg	Pro	Gln	His				

305					310					315					320
Pro	Ser	Met	Gln	Leu	Pro	Val	Pro	Pro	Gly	Gln	Pro	Pro	Xaa	Leu	Asp
				325					330					335	
Val	Cys	Val	Leu	Phe	Gly	Gly	Xaa	Xaa	Phe	Ile	Xaa	Ile			
			340					345							

```

<400> 96
Ala Pro Phe Leu Pro Lys Pro Glu Gln Arg Val Met Arg Ala Pro Gln
  1                      5                      10                      15
Glu Lys Arg Pro Gly Pro Ala Gly Gly Thr Thr Cys Gly Gln Pro Ser
                20                      25                      30
Cys Pro Gln Ala Phe Arg Gln Ala Leu Lys Arg Thr Glu Leu Pro Arg
          35                      40                      45
Ser Ala Gly Gln Trp Arg Leu Ser Pro Pro Gln Pro Ser Arg Pro Ala
      50                      55                      60
Thr Cys Val Cys Leu Thr Arg Thr His Gln Gly Phe Arg Gly Trp Glu
  65                      70                      75                      80
Leu Asn His Pro His Leu Arg Val Ile Phe Pro Ser Pro Leu Pro Ser
                85                      90                      95
Pro Pro Arg Ala Leu Pro Gly Ala Gly Lys Lys Lys Ser Lys Lys Lys
          100                      105                      110
Arg Lys Lys Lys Lys Arg Asn Lys Pro Pro Leu His Ile Met Glu Arg
      115                      120                      125
Lys Tyr Phe Cys Arg Phe Leu Phe Phe Tyr Asn Tyr Ala Trp Lys Lys
  130                      135                      140

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```
<400> 97
Met Lys Val Leu Ala Thr Ser Phe Val Leu Gly Ser Leu Gly Leu Ala
  1                      5                      10                     15
Phe Tyr Leu Pro Leu Val Val Thr Thr Pro Lys Thr Leu Ala Ile Pro
      20                      25                      30
```

76

Glu Lys Leu Gln Glu Ala Val Gly Lys Val Ile Ile Asn Ala Thr Thr
 35 40 45
 Cys Thr Val Thr Cys Gly Leu Gly Tyr Lys Glu Glu Thr Val Cys Glu
 50 55 60
 Val Gly Pro Asp Gly Val Arg Arg Lys Cys Gln Thr Gln Arg Leu Glu
 65 70 75 80
 Cys Leu Thr Asn Trp Ile Cys Gly Met Leu His Phe Thr Ile Leu Ile
 85 90 95
 Gly Lys Glu Phe Glu Leu Ser Cys Leu Ser Ser Asp Ile Leu Glu Phe
 100 105 110
 Gly Gln Glu Ala Phe Arg Phe Thr Trp Arg Leu Ala Arg Gly Val Ile
 115 120 125
 Ser Thr Asp Asp Glu Val Phe Lys Pro Phe Gln Ala Asn Ser His Phe
 130 135 140
 Val Lys Phe Lys Tyr Ala Gln Glu Tyr Asp Ser Gly Thr Tyr Arg Cys
 145 150 155 160
 Asp Val Gln Leu Val Lys Asn Leu Arg Leu Val Lys Arg Leu Tyr Phe
 165 170 175
 Gly Leu Arg Val Leu Pro Pro Asn Leu Val Asn Leu Asn Phe His Gln
 180 185 190
 Ser Leu Thr Glu Asp Gln Lys Leu Ile Asp Glu Gly Leu Glu Val Asn
 195 200 205
 Leu Asp Ser Tyr Ser Lys Pro His His Pro Lys Trp Lys Lys Lys Val
 210 215 220
 Ala Ser Ala Leu Gly Ile Gly Ile Ala Ile Gly Val Val Gly Gly Val
 225 230 235 240
 Leu Val Arg Ile Val Leu Cys Ala Leu Arg Gly Gly Leu Gln Gln
 245 250 255

<210> 98

<211> 177

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (121)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (122)

<223> Xaa equals any of the naturally occurring L-amino acids

77

<400> 98

Met Lys Val Leu Ala Thr Ser Phe Val Leu Gly Ser Leu Gly Leu Ala
 1 5 10 15

Phe Tyr Leu Pro Leu Val Val Thr Thr Pro Lys Thr Leu Ala Ile Pro
 20 25 30

Glu Lys Leu Gln Glu Ala Val Gly Lys Val Ile Ile Asn Ala Thr Thr
 35 40 45

Cys Thr Val Thr Cys Gly Leu Gly Tyr Lys Glu Glu Thr Val Cys Glu
 50 55 60

Val Gly Pro Asp Gly Val Arg Arg Lys Cys Gln Thr Arg Arg Leu Glu
 65 70 75 80

Cys Leu Thr Asn Trp Ile Cys Gly Met Leu His Phe Thr Ile Leu Ile
 85 90 95

Gly Lys Glu Phe Glu Leu Ser Cys Leu Ser Ser Asp Ile Leu Glu Phe
 100 105 110

Gly Gln Glu Ala Phe Arg Phe Thr Xaa Xaa Leu Ala Arg Gly Val Ile
 115 120 125

Ser Thr Asp Asp Glu Val Phe Lys Pro Phe Gln Ala Asn Ser His Phe
 130 135 140

Val Lys Phe Lys Tyr Ala Gln Glu Tyr Asp Ser Gly Thr Tyr Arg Cys
 145 150 155 160

Asp Val Gln Leu Val Lys Asn Leu Arg Leu Val Lys Ser Ser Ile Leu
 165 170 175

Gly

<210> 99

<211> 87

<212> PRT

<213> Homo sapiens

<400> 99

Met Thr Gly Gln Ile Pro Arg Leu Ser Lys Val Asn Leu Phe Thr Leu
 1 5 10 15

Leu Ser Leu Trp Met Glu Leu Phe Pro Ala Glu Ala Gln Arg Gln Lys
 20 25 30

Ser Gln Lys Asn Glu Glu Gly Lys His Gly Pro Leu Gly Asp Asn Glu
 35 40 45

Glu Arg Thr Arg Val Ser Thr Asp Lys Arg Gln Lys Thr Met Phe Cys
 50 55 60

Leu Phe Glu Asn Asp Cys Lys Cys Lys Ala Leu Arg Val Met Ile Arg
 65 70 75 80

78

Ser Met Ser Arg Ser Val Pro
85

<210> 100

<211> 131

<212> PRT

<213> Homo sapiens

<400> 100

Asn Ile Phe Leu Glu Trp Ile Leu Arg Arg Ile Leu Ser Leu Trp Arg
1 5 10 15

Gly Thr Phe Leu Met His Gly Arg Ala Gly Val Asn Arg Ile Ser Tyr
20 25 30

Trp Pro Ala Asp Pro Glu Ile Ser Leu Leu Thr Glu Ala Ser Ser Ser
35 40 45

Glu Asp Ala Lys Leu Asp Ala Lys Ala Val Glu Arg Leu Lys Ser Asn
50 55 60

Ser Arg Ala His Val Cys Val Leu Leu Gln Pro Leu Val Cys Tyr Met
65 70 75 80

Val Gln Phe Val Glu Glu Thr Ser Tyr Lys Cys Asp Phe Ile Gln Lys
85 90 95

Ile Thr Lys Thr Leu Pro Asp Ala Asn Thr Asp Phe Tyr Tyr Glu Cys
100 105 110

Lys Gln Glu Arg Ile Lys Glu Tyr Glu Met Leu Lys Lys Lys Lys Lys
115 120 125

Lys Lys Thr
130

<210> 101

<211> 100

<212> PRT

<213> Homo sapiens

<400> 101

Met Val Arg Thr Arg Ala Leu Phe Tyr Ile Phe Phe Gln Leu Ser Leu
1 5 10 15

Thr Ser Gly Leu Ile Glu Asp Ser Cys Ile Leu Ile Ile Ile Tyr Leu
20 25 30

Phe Phe Phe Arg Trp Cys Leu Ala Leu Ser Pro Met Leu Glu Cys Ser
35 40 45

Gly Val Thr Leu Ala His Cys Asn His His Leu Leu Gly Arg Leu Arg
50 55 60

Gln Glu Asn Arg Leu Asn Leu Gly Gly Gly Asp Cys Ser Glu Leu Arg

[illegible]

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<210> 102
<211> 94
<212> PRT
<213> Homo sapiens
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<400> 102
Met Pro His Ser Ser Leu Tyr Pro Pro Pro Phe Phe Lys Met Lys Leu
  1              5              10              15

Ile Ile Arg Val Trp Phe Ile Ile Ser Leu Phe Phe Val Gln Gly Arg
      20              25              30

Thr Asn Pro Cys Ile Leu Leu Pro Tyr Thr His Pro Gln Val Ala Leu
      35              40              45

His Leu Leu Phe Cys Ala Leu Leu Phe Ser Asp Ala Leu Gly Lys Ala
  50              55              60

Thr Ser Val Met Thr Tyr Thr Gly Phe Phe Thr His Ser Thr His Cys
  65              70              75              80

Arg Phe His Ile Ser Cys Phe Ser Leu Ser Phe Leu Ile Leu
      85              90

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<210> 103
<211> 133
<212> PRT
<213> Homo sapiens
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```

<400> 103
His Gln Ala Ile Lys Pro Gly Tyr Ser Ala Glu Asn Val Ala His Thr
  1             5             10             15

Asp His Thr Leu Gly Cys Val Thr Ile Val Trp Cys Thr Cys Trp Lys
      20             25             30

Asn Ser Ser Met Leu Leu Gly Asp Ile Ile Ser Val Gly Asn Met Pro
      35             40             45

Leu Thr Asp Phe Phe Phe Phe Leu Phe Ala Val Gly Leu Gly Gln Leu
  50             55             60

Ile Gln Gln Ser Ile Phe Phe Phe Phe Leu Ser Pro Asn Leu Asn Arg
  65             70             75             80

Ser Lys Met Cys Ser Gly Ile Pro Gly Asn Arg Cys Val Cys Lys Val
      85             90             95

```

80

Lys Asn Arg Leu Phe Arg Asn Ser Leu Phe Arg Tyr Leu His Pro Ala
 100 105 110

Ser His Val Lys Tyr Leu Ser Leu Lys Gly Leu Arg Cys Thr Ser Phe
 115 120 125

Ile Ser Tyr Phe Ser
 130

<210> 104

<211> 240

<212> PRT

<213> Homo sapiens

<400> 104

Met Thr Ser Val Ser Gln Ala Ser Leu Asp Val Ser Met Ile Ile Ile
 1 5 10 15

Ile Ser Leu Gly Ala Ile Cys Ala Val Leu Leu Val Ile Met Val Leu
 20 25 30

Phe Ala Thr Arg Cys Asn Arg Glu Lys Lys Asp Thr Arg Ser Tyr Asn
 35 40 45

Cys Arg Val Ala Glu Ser Thr Tyr Gln His His Pro Lys Arg Pro Ser
 50 55 60

Arg Gln Ile His Lys Gly Asp Ile Thr Leu Val Pro Thr Ile Asn Gly
 65 70 75 80

Thr Leu Pro Ile Arg Ser His His Arg Ser Ser Pro Ser Ser Ser Pro
 85 90 95

Thr Leu Glu Arg Gly Gln Met Gly Ser Arg Gln Ser His Asn Ser His
 100 105 110

Gln Ser Leu Asn Ser Leu Val Thr Ile Ser Ser Asn His Val Pro Glu
 115 120 125

Asn Phe Ser Leu Glu Leu Thr His Ala Thr Pro Ala Val Glu Val Ser
 130 135 140

Gln Leu Leu Ser Met Leu His Gln Gly Gln Tyr Gln Pro Arg Pro Ser
 145 150 155 160

Phe Arg Gly Asn Lys Tyr Ser Arg Ser Tyr Arg Tyr Ala Leu Gln Asp
 165 170 175

Met Asp Lys Phe Ser Leu Lys Asp Ser Gly Arg Gly Asp Ser Glu Ala
 180 185 190

Gly Asp Ser Asp Tyr Asp Leu Gly Arg Asp Ser Pro Ile Asp Arg Leu
 195 200 205

Leu Gly Glu Gly Phe Ser Asp Leu Phe Leu Thr Asp Gly Arg Ile Pro
 210 215 220

81

Ala Ser Tyr Glu Thr Leu His Gly Gly Val Gln Gly Pro Gly Thr Leu
 225 230 235 240

<210> 105
 <211> 180
 <212> PRT
 <213> Homo sapiens

<400> 105
 Met Thr Ser Val Ser Gln Ala Ser Leu Asp Val Ser Met Ile Ile Ile
 1 5 10 15
 Ile Ser Leu Gly Ala Ile Cys Ala Val Leu Leu Val Ile Met Val Leu
 20 25 30
 Phe Ala Thr Arg Cys Asn Arg Glu Lys Lys Asp Thr Arg Ser Tyr Asn
 35 40 45
 Cys Arg Val Ala Glu Ser Thr Tyr Gln His His Pro Lys Arg Pro Ser
 50 55 60
 Arg Gln Ile His Lys Gly Asp Ile Thr Leu Val Pro Thr Ile Asn Gly
 65 70 75 80
 Thr Leu Pro Ile Arg Ser His His Arg Ser Ser Pro Ser Ser Ser Pro
 85 90 95
 Thr Leu Glu Arg Gly Gln Met Gly Ser Arg Gln Ser His Asn Ser His
 100 105 110
 Gln Ser Leu Asn Ser Leu Val Thr Ile Ser Ser Asn His Val Pro Glu
 115 120 125
 Asn Phe Ser Leu Glu Leu Thr His Ala Thr Pro Ala Val Glu Arg Leu
 130 135 140
 Ser Ala Ser Phe Asn Ala Ser Pro Gly Ala Ile Ser Ala Lys Thr Lys
 145 150 155 160
 Phe Ser Arg Lys Gln Ile Phe Gln Glu Leu Gln Ile Cys Pro Ser Arg
 165 170 175
 His Gly Gln Ile
 180

<210> 106
 <211> 93
 <212> PRT
 <213> Homo sapiens

<400> 106
 Met Ile Val Asn Ile Ser His Glu Ile Trp Trp Phe Tyr Lys Gly Lys
 1 5 10 15

82

Val Pro Leu His Met Leu Thr Cys Leu Leu Pro Cys Lys Thr Cys Leu
 20 25 30

Ala Pro Pro Ser Pro Ser Ser Val Thr Val Arg Pro Pro Gln Pro Cys
 35 40 45

Glu Thr Val Ser Pro Leu Lys Leu Phe Phe Phe Ile Asn Tyr Pro Val
 50 55 60

Leu His Met Ser Leu Leu Thr Val Arg Lys Trp Thr Asn Thr Leu Gly
 65 70 75 80

His Glu Gly Gly Ala Leu Ile Asn Gly Ile Ser Ala Leu
 85 90

<210> 107
 <211> 59
 <212> PRT
 <213> Homo sapiens

<400> 107
 Glu Glu His Gly Ile Thr Ser Val Ile Phe Leu Pro Gln Val His Asn
 1 5 10 15

Leu Asn Leu Ile Ile Arg Lys His Gln Thr Asn Pro Asn Gln Glu Thr
 20 25 30

Leu Tyr Lys Ile Met Thr Cys Asp Pro Gln Asn Leu Gln Gly His Glu
 35 40 45

Gln Gln Gly Lys Thr Glu Asp Lys Cys Thr Val
 50 55

<210> 108
 <211> 200
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (140)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (155)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (165)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE

<222> (173)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (194)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (196)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 108

Met	Ala	Val	Tyr	Val	Gly	Met	Leu	Arg	Leu	Gly	Arg	Leu	Cys	Ala	Gly
1				5					10					15	

Ser	Ser	Gly	Val	Leu	Gly	Ala	Arg	Ala	Ala	Leu	Ser	Arg	Ser	Trp	Gln
		20					25						30		

Glu	Ala	Arg	Leu	Gln	Gly	Val	Arg	Phe	Leu	Ser	Ser	Arg	Glu	Val	Asp
		35					40					45			

Arg	Met	Val	Ser	Thr	Pro	Ile	Gly	Gly	Leu	Ser	Tyr	Val	Gln	Gly	Cys
	50					55					60				

Thr	Lys	Lys	His	Leu	Asn	Ser	Lys	Thr	Val	Gly	Gln	Cys	Leu	Glu	Thr
65					70					75					80

Thr	Ala	Gln	Arg	Val	Pro	Glu	Arg	Glu	Ala	Leu	Val	Val	Leu	His	Glu
				85					90					95	

Asp	Val	Arg	Leu	Thr	Phe	Ala	Gln	Leu	Lys	Glu	Glu	Val	Asp	Lys	Ala
			100					105					110		

Ala	Ser	Gly	Leu	Leu	Ser	Ile	Gly	Leu	Cys	Lys	Gly	Asp	Arg	Leu	Gly
		115					120					125			

Met	Trp	Gly	Pro	Asn	Ser	Tyr	Ala	Trp	Val	Leu	Xaa	Gln	Leu	Ala	Thr
	130					135						140			

Gly	Gln	Ala	Gly	Ile	Ile	Leu	Val	Ser	Val	Xaa	Pro	Ala	Tyr	Gln	Ala
145					150					155					160

Met	Glu	Trp	Ser	Xaa	Ser	Ser	Lys	Lys	Trp	Ala	Ser	Xaa	Ala	Leu	Val
				165					170					175	

Val	Pro	Lys	Gln	Phe	Lys	Thr	Lys	His	Asn	Thr	Thr	Phe	Leu	Lys	Gln
			180					185					190		

Ile	Xaa	Pro	Xaa	Trp	Arg	Met	Pro
		195					200

<210> 109

<211> 100

<212> PRT

<213> Homo sapiens

<220>
 <221> SITE
 <222> (3)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (12)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (57)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (62)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (80)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 109
 Met Ala Xaa Tyr Val Gly Met Leu Arg Leu Gly Xaa Leu Cys Ala Gly
 1 5 10 15
 Ser Ser Gly Val Leu Gly Ala Arg Ala Ala Leu Ser Arg Ser Trp Gln
 20 25 30
 Glu Ala Arg Leu Gln Gly Val Arg Phe Leu Ser Ser Arg Glu Val Gly
 35 40 45
 Ser His Gly Leu His Ala His Arg Xaa Ala Ser Ala Thr Xaa Arg Gly
 50 55 60
 Ala Pro Lys Ser Ile Leu Thr Ala Arg Leu Trp Ala Ser Ala Trp Xaa
 65 70 75 80
 Pro Gln His Arg Gly Ser Gln Asn Glu Arg Pro Trp Ser Ser Ser Met
 85 90 95
 Lys Thr Ser Gly
 100

<210> 110
 <211> 87
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (71)
 <223> Xaa equals any of the naturally occurring L-amino acids

85

<220>

<221> SITE

<222> (86)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 110

Met	Val	Val	Met	Ala	Ser	Leu	Gln	Val	Glu	Pro	Ala	Val	Gly	Lys	Glu
1				5					10					15	

Gln	Leu	Arg	Glu	Arg	Gln	Gly	Pro	Glu	Leu	Leu	Gly	Trp	Val	Ala	Gly
			20					25					30		

Leu	Ala	Phe	Val	Cys	Leu	Phe	Ala	Cys	Val	Gly	Val	Gly	Val	Ala	Pro
		35					40					45			

Cys	His	Ser	Phe	Asp	Ser	Glu	Ala	Ala	Ser	Phe	Leu	Leu	Leu	Tyr	Ser
	50					55					60				

Trp	Cys	Thr	Pro	Arg	Leu	Xaa	Ser	Trp	Leu	Arg	Asp	Thr	Pro	Ser	Pro
65					70					75					80

Leu	Ala	Ser	Gly	Thr	Xaa	Pro
					85	

<210> 111

<211> 49

<212> PRT

<213> Homo sapiens

<400> 111

Val	Arg	Ala	Met	Phe	Gly	Phe	Leu	Ala	Cys	Val	Ser	Ser	Leu	Arg	Val
1				5					10					15	

Met	Ala	Ser	Ser	Ser	Ser	His	Val	Thr	Ser	Glu	Asp	Met	Ile	Leu	Phe
			20					25					30		

Leu	Ile	Ser	Cys	Gly	Ile	Tyr	Val	Pro	His	Phe	Leu	Tyr	Pro	Val	Asp
		35					40					45			

Arg

<210> 112

<211> 141

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (140)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 112

Met	Gly	Pro	Arg	Gly	Cys	Ala	Leu	Ala	His	Ser	Leu	Leu	Pro	Leu	Leu
1				5					10					15	

86

Cys Gln His Val Trp Thr Ser Pro Arg Tyr Cys Arg Gln Cys Thr Arg
 20 25 30
 Glu Pro Arg His Cys Cys Pro Ala Pro Ala Ser Ala Gly Val Gln Tyr
 35 40 45
 Met Cys Ala Tyr Gly Cys His His Pro Thr Phe Ala Gly Val Tyr Thr
 50 55 60
 Pro Ser His Thr Thr Val Ala Thr Ser Ile Cys Thr Gln Thr Pro Pro
 65 70 75 80
 His Gln Cys Cys Trp Ser Glu His Thr His Val Val Ser Thr Thr Pro
 85 90 95
 Leu Leu Pro Ala Tyr Met His Met Ser Met Asp Pro Ala Ala Thr Thr
 100 105 110
 Gln Met Lys Cys Phe Cys Arg His Pro Ile Arg Ala Phe Leu Pro Val
 115 120 125
 Glu Trp Glu His Leu Ser Pro Phe Asn Thr Ala Xaa Ala
 130 135 140

<210> 113
 <211> 49
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (1)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (2)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 113
 Xaa Xaa Tyr Asp Glu Lys Leu Ile Phe Ile Gln Ile Leu Gln Thr Lys
 1 5 10 15
 Ala Thr Asp Lys Tyr Ser Glu Gln Val Ser Gln Val Gly Pro Gly Ala
 20 25 30
 Val Leu Thr Pro Val Ile Pro Ala Leu Trp Glu Ala Glu Ala Gly Gly
 35 40 45
 Ser

<210> 114
 <211> 125
 <212> PRT

87

<213> Homo sapiens

<400> 114

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Met Val Pro Ile Phe Leu Leu Lys Cys Leu Leu Leu His Val Pro Leu
 1           5           10           15

Cys Met Ser Ser Asn Leu Ser Phe His Ser Ser His His Leu His Ile
          20           25           30

Phe Leu Pro Ser Phe Ser Ser His Leu Pro Arg Pro Leu Tyr Ile Pro
          35           40           45

Pro Leu Ser Pro Phe Tyr Ile Phe Ser Ile Ser Pro His Ile Phe Pro
          50           55           60

Leu Cys Pro His Leu Cys Ile Pro Pro Asn Phe Pro Ser Ile Tyr Leu
 65           70           75           80

Phe Tyr Ser Pro Phe Pro Pro Cys Ile Leu Cys Val Pro Pro Ile Leu
          85           90           95

Leu Tyr Ile Ile Leu Pro Lys Ile Phe Thr Ser Pro Ile Leu Ile Ser
          100          105          110

Pro Ser Pro Leu Ser Pro Asn Ile Phe Ile Ser Val Pro
          115          120          125

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<210> 115

<211> 52

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (46)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 115

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Met Val Pro Ile Phe Leu Leu Lys Cys Leu Leu Leu His Val Pro Leu
 1           5           10           15

Cys Met Ser Ser Asn Leu Ser Phe His Ser Ser His His Leu His Ile
          20           25           30

Phe Leu Pro Ser Phe Ser Ser His Leu Pro Arg Pro Leu Xaa Ile Pro
          35           40           45

Pro Leu Ser Pro
          50

```

<210> 116

<211> 1134

<212> PRT

<213> Homo sapiens

<400> 116

88

Val Leu Phe Arg Pro Gln Ala Gln Arg Pro Pro Ser Cys Val Gly Gly
 1 5 10 15
 Ser Ala Val Arg Arg Trp Gln Gly Gln Pro Gln Pro Gln Arg Pro Gly
 20 25 30
 Glu Glu Lys Ala Ala Ala Ala Ile Leu Gly Gly Pro Gly Gly Gly Glu
 35 40 45
 Glu Glu Lys Glu Glu Gly Gly Gly Arg Ala Trp Leu Arg Leu Leu Glu
 50 55 60
 Glu Leu Ala Ala Ala Arg Pro Gly Glu Pro Ala Leu Met Ser Ser Ser
 65 70 75 80
 Pro Leu Ser Lys Lys Arg Arg Val Ser Gly Pro Asp Pro Lys Pro Gly
 85 90 95
 Ser Asn Cys Ser Pro Ala Gln Ser Val Leu Ser Glu Val Pro Ser Val
 100 105 110
 Pro Thr Asn Gly Met Ala Lys Asn Gly Ser Glu Ala Asp Ile Asp Glu
 115 120 125
 Gly Leu Tyr Ser Arg Gln Leu Tyr Val Leu Gly His Glu Ala Met Lys
 130 135 140
 Arg Leu Gln Thr Ser Ser Val Leu Val Ser Gly Leu Arg Gly Leu Gly
 145 150 155 160
 Val Glu Ile Ala Lys Asn Ile Ile Leu Gly Gly Val Lys Ala Val Thr
 165 170 175
 Leu His Asp Gln Gly Thr Ala Gln Trp Ala Asp Leu Ser Ser Gln Phe
 180 185 190
 Tyr Leu Arg Glu Glu Asp Ile Gly Lys Asn Arg Ala Glu Val Ser Gln
 195 200 205
 Pro Arg Leu Ala Glu Leu Asn Ser Tyr Val Pro Val Thr Ala Tyr Thr
 210 215 220
 Gly Pro Leu Val Glu Asp Phe Leu Ser Gly Phe Gln Val Val Val Leu
 225 230 235 240
 Thr Asn Thr Pro Leu Glu Asp Gln Leu Arg Val Gly Glu Phe Cys His
 245 250 255
 Asn Arg Gly Ile Lys Leu Val Val Ala Asp Thr Arg Gly Leu Phe Gly
 260 265 270
 Gln Leu Phe Cys Asp Phe Gly Glu Glu Met Ile Leu Thr Asp Ser Asn
 275 280 285
 Gly Glu Gln Pro Leu Ser Ala Met Val Ser Met Val Thr Lys Asp Asn
 290 295 300
 Pro Gly Val Val Thr Cys Leu Asp Glu Ala Arg His Gly Phe Glu Ser

89

305		310		315		320
Gly Asp Phe Val Ser Phe Ser Glu Val Gln Gly Met Val Glu Leu Asn						
		325		330		335
Gly Asn Gln Pro Met Glu Ile Lys Val Leu Gly Pro Tyr Thr Phe Ser						
		340		345		350
Ile Cys Asp Thr Ser Asn Phe Ser Asp Tyr Ile Arg Gly Gly Ile Val						
		355		360		365
Ser Gln Val Lys Val Pro Lys Lys Ile Ser Phe Lys Ser Leu Val Ala						
		370		375		380
Ser Leu Ala Glu Pro Asp Phe Val Val Thr Asp Phe Ala Lys Phe Ser						
		385		390		395
Arg Pro Ala Gln Leu His Ile Gly Phe Gln Ala Leu His Gln Phe Cys						
		405		410		415
Ala Gln His Gly Arg Pro Pro Arg Pro Arg Asn Glu Glu Asp Ala Ala						
		420		425		430
Glu Leu Val Ala Leu Ala Gln Ala Val Asn Ala Arg Ala Leu Pro Ala						
		435		440		445
Val Gln Gln Asn Asn Leu Asp Glu Asp Leu Ile Arg Lys Leu Ala Tyr						
		450		455		460
Val Ala Ala Gly Asp Leu Ala Pro Ile Asn Ala Phe Ile Gly Gly Leu						
		465		470		475
Ala Ala Gln Glu Val Met Lys Ala Cys Ser Gly Lys Phe Met Pro Ile						
		485		490		495
Met Gln Trp Leu Tyr Phe Asp Ala Leu Glu Cys Leu Pro Glu Asp Lys						
		500		505		510
Glu Val Leu Thr Glu Asp Lys Cys Leu Gln Arg Gln Asn Arg Tyr Asp						
		515		520		525
Gly Gln Val Ala Val Phe Gly Ser Asp Leu Gln Glu Lys Leu Gly Lys						
		530		535		540
Gln Lys Tyr Phe Leu Val Gly Ala Gly Ala Ile Gly Cys Glu Leu Leu						
		545		550		555
Lys Asn Phe Ala Met Ile Gly Leu Gly Cys Gly Glu Gly Gly Glu Ile						
		565		570		575
Ile Val Thr Asp Met Asp Thr Ile Glu Lys Ser Asn Leu Asn Arg Gln						
		580		585		590
Phe Leu Phe Arg Pro Trp Asp Val Thr Lys Leu Lys Ser Asp Thr Ala						
		595		600		605
Ala Ala Ala Val Arg Gln Met Asn Pro His Ile Arg Val Thr Ser His						
		610		615		620

Gln Asn Arg Val Gly Pro Asp Thr Glu Arg Ile Tyr Asp Asp Asp Phe
 625 630 635 640
 Phe Gln Asn Leu Asp Gly Val Ala Asn Ala Leu Asp Asn Val Asp Ala
 645 650 655
 Arg Met Tyr Met Asp Arg Arg Cys Val Tyr Tyr Arg Lys Pro Leu Leu
 660 665 670
 Glu Ser Gly Thr Leu Gly Thr Lys Gly Asn Val Gln Val Val Ile Pro
 675 680 685
 Phe Leu Thr Glu Ser Tyr Ser Ser Ser Gln Asp Pro Pro Glu Lys Ser
 690 695 700
 Ile Pro Ile Cys Thr Leu Lys Asn Phe Pro Asn Ala Ile Glu His Thr
 705 710 715 720
 Leu Gln Trp Ala Arg Asp Glu Phe Glu Gly Leu Phe Lys Gln Pro Ala
 725 730 735
 Glu Asn Val Asn Gln Tyr Leu Thr Asp Pro Lys Phe Val Glu Arg Thr
 740 745 750
 Leu Arg Leu Ala Gly Thr Gln Pro Leu Glu Val Leu Glu Ala Val Gln
 755 760 765
 Arg Ser Leu Val Leu Gln Arg Pro Gln Thr Trp Ala Asp Cys Val Thr
 770 775 780
 Trp Ala Cys His His Trp His Thr Gln Tyr Ser Asn Asn Ile Arg Gln
 785 790 795 800
 Leu Leu His Asn Phe Pro Pro Asp Gln Leu Thr Ser Ser Gly Ala Pro
 805 810 815
 Phe Trp Ser Gly Pro Lys Arg Cys Pro His Pro Leu Thr Phe Asp Val
 820 825 830
 Asn Asn Pro Leu His Leu Asp Tyr Val Met Ala Ala Ala Asn Leu Phe
 835 840 845
 Ala Gln Thr Tyr Gly Leu Thr Gly Ser Gln Asp Arg Ala Ala Val Ala
 850 855 860
 Thr Phe Leu Gln Ser Val Gln Val Pro Glu Phe Thr Pro Lys Ser Gly
 865 870 875 880
 Val Lys Ile His Val Ser Asp Gln Glu Leu Gln Ser Ala Asn Ala Ser
 885 890 895
 Val Asp Asp Ser Arg Leu Glu Glu Leu Lys Ala Thr Leu Pro Ser Pro
 900 905 910
 Asp Lys Leu Pro Gly Phe Lys Met Tyr Pro Ile Asp Phe Glu Lys Asp
 915 920 925

Asp Asp Ser Asn Phe His Met Asp Phe Ile Val Ala Ala Ser Asn Leu
 930 935 940

Arg Ala Glu Asn Tyr Asp Ile Pro Ser Ala Asp Arg His Lys Ser Lys
 945 950 955 960

Leu Ile Ala Gly Lys Ile Ile Pro Ala Ile Ala Thr Thr Thr Ala Ala
 965 970 975

Val Val Gly Leu Val Cys Leu Glu Leu Tyr Lys Val Val Gln Gly His
 980 985 990

Arg Gln Leu Asp Ser Tyr Lys Asn Gly Phe Leu Asn Leu Ala Leu Pro
 995 1000 1005

Phe Phe Gly Phe Ser Glu Pro Leu Ala Ala Pro Arg His Gln Tyr
 1010 1015 1020

Tyr Asn Gln Glu Trp Thr Leu Trp Asp Arg Phe Glu Val Gln Gly
 1025 1030 1035

Leu Gln Pro Asn Gly Glu Glu Met Thr Leu Lys Gln Phe Leu Asp
 1040 1045 1050

Tyr Phe Lys Thr Glu His Lys Leu Glu Ile Thr Met Leu Ser Gln
 1055 1060 1065

Gly Val Ser Met Leu Tyr Ser Phe Phe Met Pro Ala Ala Lys Leu
 1070 1075 1080

Lys Glu Arg Leu Asp Gln Pro Met Thr Glu Ile Val Ser Arg Val
 1085 1090 1095

Ser Lys Arg Lys Leu Gly Arg His Val Arg Ala Leu Val Leu Glu
 1100 1105 1110

Leu Cys Cys Asn Asp Glu Ser Gly Glu Asp Val Glu Val Pro Tyr
 1115 1120 1125

Val Arg Tyr Thr Ile Arg
 1130

<210> 117

<211> 215

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (116)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 117

Met Glu Leu Ser Cys Pro Gly Ser Arg Cys Pro Val Gln Glu Gln Arg
 1 5 10 15

Ala Arg Trp Glu Arg Lys Arg Ala Cys Thr Ala Arg Glu Leu Leu Glu

20

25

30

Leu Gly Ile Leu Lys Ala Lys Gly Thr Leu Arg Pro Pro Glu Arg Gln
50 55 60

Ala Leu Phe Gly Ser Trp Glu Leu Ile Tyr Gly Ala Ser Gln Glu Leu
65 70 75 80

Leu Pro Tyr Leu Glu Gly Gly Cys Trp Gly Gln Gly Leu Glu Gly Phe
85 90 95

Cys Arg His Leu Glu Leu Tyr Asn Gln Phe Ala Ala Asn Ser Glu Arg
100 105 110

Ser Gln Thr Xaa Leu Gln Glu Gln Leu Lys Lys Asn Lys Gly Phe Arg
115 120 125

Lys Phe Val Arg Leu Gln Glu Gly Arg Pro Glu Phe Gly Gly Leu Gln
130 135 140

Leu Gln Asp Leu Leu Pro Leu Pro Leu Gln Arg Leu Gln Gln Tyr Glu
145 150 155 160

Asn Leu Val Val Ala Leu Ala Glu Asn Thr Gly Pro Asn Ser Pro Asp
165 170 175

His Gln Gln Leu Thr Arg Arg Phe Leu Leu Leu Gly Asn Ala Gly Trp
180 185 190

Arg Leu Pro Leu Leu Tyr Ser Phe Leu Ile Leu Thr Ser Asn Asn Val
195 200 205

Trp Tyr Asp Pro Ile Phe His
210 215

<210> 118

<211> 69

<212> PRT

<213> Homo sapiens

<400> 118

Glu Ile Leu Leu Lys Lys Lys Asn Gln Glu Thr Lys Ser Asn Pro Thr
1 5 10 15

Lys Pro Gln Met Asn Gln Pro Leu Thr Gln Met Arg Gly Phe Gly Thr
20 25 30

Asp Lys Leu Cys Ala Val Ser Met Ala Arg His Leu Ser Arg Leu Gln
35 40 45

Leu Cys Lys Cys Gly Tyr Phe Tyr Val Val Tyr Ser Phe Tyr His Leu
50 55 60

Phe Phe His Trp Ile

65

<210> 119

<211> 98

<212> PRT

<213> Homo sapiens

<400> 119

Met	Cys	Met	Arg	Leu	Cys	Ala	Ala	Leu	Leu	Pro	Ala	Pro	Cys	Thr	Leu
1				5					10					15	

Arg	Ala	Ser	Trp	Gly	Val	Arg	Gly	Ala	Gln	Trp	Gly	Phe	Ser	Ser	Leu
			20					25					30		

His	Glu	Pro	Gly	Asp	Pro	Arg	Gly	Gly	Ser	Ile	Trp	Asp	Glu	Pro	Pro
		35					40					45			

Pro	Pro	Asn	Ala	Gln	Ala	Ser	Pro	Gln	Asp	Pro	Gly	Gly	Gly	His	His
	50					55					60				

Ser	Gly	Lys	Pro	Gly	Val	Gly	Val	Gly	Phe	Gly	Leu	Ser	Thr	Phe	Leu
65					70					75					80

Leu	Gln	Ile	Pro	Pro	Thr	His	Pro	Ser	Pro	Lys	Ser	Ser	Pro	Leu	Ala
				85					90					95	

Leu Ala

<210> 120

<211> 1

<212> PRT

<213> Homo sapiens

<400> 120

Met

1

<210> 121

<211> 303

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (176)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (177)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (179)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (192)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (294)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (297)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (302)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 121

Met	Ser	Ser	Asn	Thr	Met	Leu	Gln	Lys	Thr	Leu	Leu	Ile	Leu	Ile	Ser
1				5					10					15	

Phe	Ser	Val	Val	Thr	Trp	Met	Ile	Phe	Ile	Ile	Ser	Gln	Asn	Phe	Thr
		20					25					30			

Lys	Leu	Trp	Ser	Ala	Leu	Asn	Leu	Ser	Ile	Ser	Val	His	Tyr	Trp	Asn
	35					40						45			

Asn	Ser	Ala	Lys	Ser	Leu	Phe	Pro	Lys	Thr	Ser	Leu	Ile	Pro	Leu	Lys
	50					55					60				

Pro	Leu	Thr	Glu	Thr	Glu	Leu	Arg	Ile	Lys	Glu	Ile	Ile	Glu	Lys	Leu
65					70					75					80

Asp	Gln	Gln	Ile	Pro	Pro	Arg	Pro	Phe	Thr	His	Val	Asn	Thr	Thr	Thr
				85					90					95	

Ser	Ala	Thr	His	Ser	Thr	Ala	Thr	Ile	Leu	Asn	Pro	Arg	Asp	Thr	Tyr
		100						105					110		

Cys	Arg	Gly	Asp	Gln	Leu	Asp	Ile	Leu	Leu	Glu	Val	Arg	Asp	His	Leu
	115					120						125			

Gly	Gln	Arg	Lys	Gln	Tyr	Gly	Gly	Asp	Phe	Leu	Arg	Ala	Arg	Met	Ser
130						135					140				

Ser	Pro	Ala	Leu	Thr	Ala	Gly	Ala	Ser	Gly	Lys	Val	Met	Asp	Phe	Asn
145					150					155					160

Asn	Gly	Thr	Tyr	Leu	Val	Ser	Phe	Thr	Leu	Phe	Trp	Glu	Gly	Gln	Xaa
				165					170					175	

Xaa	Leu	Xaa	Leu	Leu	Leu	Ile	His	Pro	Ser	Glu	Gly	Ala	Ser	Ala	Xaa
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

95

	180		185		190										
Trp	Arg	Ala	Arg	Asn	Gln	Gly	Tyr	Asp	Lys	Ile	Ile	Phe	Lys	Gly	Lys
	195						200					205			
Phe	Val	Asn	Gly	Thr	Ser	His	Val	Phe	Thr	Glu	Cys	Gly	Leu	Thr	Leu
	210					215					220				
Asn	Ser	Asn	Ala	Glu	Leu	Cys	Glu	Tyr	Leu	Asp	Asp	Arg	Asp	Gln	Glu
225					230					235					240
Ala	Phe	Tyr	Cys	Met	Lys	Pro	Gln	His	Met	Pro	Cys	Glu	Ala	Leu	Thr
				245					250					255	
Tyr	Met	Thr	Thr	Arg	Asn	Arg	Glu	Val	Ser	Tyr	Leu	Thr	Asp	Lys	Glu
			260					265					270		
Asn	Ser	Leu	Phe	His	Arg	Ser	Lys	Val	Gly	Val	Glu	Met	Met	Lys	Asp
	275						280					285			
Arg	Lys	His	Ile	Asp	Xaa	Thr	Asn	Xaa	Asn	Lys	Arg	Glu	Xaa	Ile	
	290					295					300				

<210> 122

<211> 191

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (180)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (181)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (190)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 122

Met	Ser	Ser	Asn	Thr	Met	Leu	Gln	Lys	Thr	Leu	Leu	Ile	Leu	Ile	Ser
1				5					10					15	

Phe	Ser	Val	Val	Thr	Trp	Met	Ile	Phe	Ile	Ile	Ser	Gln	Asn	Phe	Thr
			20					25					30		

Lys	Leu	Trp	Ser	Ala	Leu	Asn	Leu	Ser	Ile	Ser	Val	His	Tyr	Trp	Asn
		35				40						45			

Asn	Ser	Ala	Lys	Ser	Leu	Phe	Pro	Lys	Thr	Ser	Leu	Ile	Pro	Leu	Lys
	50					55						60			

Pro	Leu	Thr	Glu	Thr	Glu	Leu	Arg	Ile	Lys	Glu	Ile	Ile	Glu	Lys	Leu
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

96

65		70		75		80									
Asp	Gln	Gln	Ile	Pro	Pro	Arg	Pro	Phe	Thr	His	Val	Asn	Thr	Thr	Thr
				85					90					95	
Ser	Ala	Thr	His	Ser	Thr	Ala	Thr	Ile	Leu	Asn	Pro	Arg	Asp	Thr	Tyr
			100					105					110		
Cys	Arg	Gly	Asp	Gln	Leu	Asp	Ile	Leu	Leu	Glu	Val	Arg	Asp	His	Leu
		115					120					125			
Gly	Gln	Arg	Lys	Gln	Tyr	Gly	Gly	Asp	Phe	Leu	Arg	Ala	Arg	Met	Ser
	130					135					140				
Phe	Pro	Ala	Leu	Thr	Ala	Gly	Ala	Ser	Gly	Lys	Val	Met	Asp	Phe	Thr
145					150					155					160
Met	Ala	Pro	Thr	Trp	Gln	Leu	His	Ser	Gly	Leu	Gly	Gly	Pro	Gly	Leu
				165					170					175	
Pro	Gly	Ser	Xaa	Xaa	Tyr	Ser	Pro	Gln	Val	Glu	Gly	Ala	Xaa	Gly	
			180					185					190		

<210> 123

<211> 165

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (2)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (5)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (7)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (28)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (33)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 123

Asn	Xaa	Phe	Ala	Xaa	Trp	Xaa	Gln	Lys	Asp	Thr	Leu	Arg	Ile	Gln	Trp
1				5					10					15	

97

Lys Lys His Ser Tyr Pro Phe Val Thr Phe Gln Xaa Tyr Ser Leu Ile
 20 25 30
 Xaa His Asp Tyr Ile Pro Arg Glu Ile Asp Arg Leu Ser Gly Asp Lys
 35 40 45
 Asn Thr Ala Ile Val Ile Thr Phe Gly Gln His Phe Arg Pro Phe Pro
 50 55 60
 Ile Asp Ile Phe Ile Arg Arg Ala Ile Gly Val Gln Lys Ala Ile Glu
 65 70 75 80
 Arg Leu Phe Leu Arg Ser Pro Ala Thr Lys Val Ile Ile Lys Thr Glu
 85 90 95
 Asn Ile Arg Glu Met His Ile Glu Thr Glu Arg Phe Gly Asp Phe His
 100 105 110
 Gly Tyr Ile His Tyr Leu Ile Met Lys Asp Ile Phe Lys Asp Leu Asn
 115 120 125
 Val Gly Ile Ile Asp Ala Trp Asp Met Thr Ile Ala Tyr Gly Thr Asp
 130 135 140
 Thr Ile His Pro Pro Asp His Val Ile Gly Asn Gln Ile Asn Met Phe
 145 150 155 160
 Leu Asn Tyr Ile Cys
 165

<210> 124
 <211> 148
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (3)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (37)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (41)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (43)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE

<222> (99)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (121)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 124

Gly Phe Xaa Ala Ala Ala Ala Ala Ala Val Val Ala Ala Ala Ala
1 5 10 15

Ala Ala Ser Val Glu Gly Arg Gln Pro Pro Gly Leu Gly Ala Val Gly
20 25 30

Pro Ala Gly Arg Xaa Ala Gly Ser Xaa Gly Xaa Arg Met Pro Ala Gly
35 40 45

Arg Val Ala Gly Ala Val Thr Gly Leu Gly Val Ser Trp Leu Arg Gly
50 55 60

Lys Asn Ser Gly Val Pro Gly Ala Ala Leu Pro Pro Ala Ala Pro Ser
65 70 75 80

Val Ala Ser Leu Val Ala His Ser Gly Pro Ala Val Gly Pro Pro Leu
85 90 95

Ser Pro Xaa Ser Val Pro Gln Gly Gly Tyr Ser Lys Ser Gly Leu Pro
100 105 110

Leu Gln Asp Ala Gly Ser Pro Trp Xaa His Cys Arg Gly Thr Asp Cys
115 120 125

Gly Ser Ser Met Leu Asn Gly Val Glu Ala Gly Leu Ala Ala Ala Ala
130 135 140

Ser Cys Cys His
145

<210> 125

<211> 124

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (63)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (86)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 125

Met Val Val Ala Val Leu Leu Gly Phe Val Ala Met Val Leu Ser Val
1 5 10 15

99

Val Gly Met Lys Cys Thr Arg Val Gly Asp Ser Asn Pro Ile Ala Lys
 20 25 30
 Gly Arg Val Ala Ile Ala Gly Gly Ala Leu Phe Ile Leu Ala Gly Leu
 35 40 45
 Cys Thr Leu Thr Ala Val Ser Trp Tyr Ala Thr Leu Val Thr Xaa Glu
 50 55 60
 Phe Phe Asn Pro Ser Thr Pro Val Asn Ala Arg Tyr Glu Phe Gly Pro
 65 70 75 80
 Ala Leu Phe Val Gly Xaa Asp Ser Ala Gly Leu Ala Val Leu Ser Gly
 85 90 95
 Ser Phe Leu Cys Cys Thr Cys Pro Glu Pro Glu Arg Pro Asn Ser Ser
 100 105 110
 Pro Gln Ala Leu Ser Ala Trp Thr Leu Cys Cys Cys
 115 120

<210> 126
 <211> 246
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (30)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (212)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (220)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (243)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 126
 Met Ala Ser Ala Val Arg Gly Ser Arg Pro Trp Pro Arg Leu Gly Leu
 1 5 10 15
 Gln Leu Gln Phe Ala Ala Leu Leu Leu Gly Thr Leu Ser Xaa Gln Val
 20 25 30
 His Thr Leu Arg Pro Glu Asn Leu Leu Leu Val Ser Thr Leu Asp Gly
 35 40 45

100

Ser Leu His Ala Leu Ser Lys Gln Thr Gly Asp Leu Lys Trp Thr Leu
 50 55 60
 Arg Asp Asp Pro Val Ile Glu Gly Pro Met Tyr Val Thr Glu Met Ala
 65 70 75 80
 Phe Leu Ser Asp Pro Ala Asp Gly Ser Leu Tyr Ile Leu Gly Thr Gln
 85 90 95
 Lys Gln Gln Gly Leu Met Lys Leu Pro Phe Thr Ile Pro Glu Leu Val
 100 105 110
 His Ala Ser Pro Cys Arg Ser Ser Asp Gly Val Phe Tyr Thr Gly Arg
 115 120 125
 Lys Gln Asp Ala Trp Phe Val Val Asp Pro Glu Ser Gly Glu Thr Gln
 130 135 140
 Met Thr Leu Thr Thr Glu Gly Pro Ser Thr Pro Arg Leu Tyr Ile Gly
 145 150 155 160
 Arg Thr Gln Tyr Thr Val Thr Met His Asp Pro Arg Ala Pro Ala Leu
 165 170 175
 Arg Trp Asn Thr Thr Tyr Arg Arg Tyr Ser Thr Pro Pro Met Asp Gly
 180 185 190
 Ser Thr Gly Lys Tyr Met Ser Gln Leu Gly Val Leu Arg Glu Gly Pro
 195 200 205
 Ala Ala His Xaa Gly Thr Pro Gly Ser Gly Thr Xaa Leu Leu Asp Thr
 210 215 220
 Arg Asn Leu Gly Arg Ala Leu Gly Asn Gly Pro Ala Thr Pro Leu Gly
 225 230 235 240
 Thr Lys Xaa Arg Ala Trp
 245

<210> 127

<211> 452

<212> PRT

<213> Homo sapiens

<400> 127

Met Glu Leu Ala Leu Arg Arg Ser Pro Val Pro Arg Trp Leu Leu Leu
 1 5 10 15
 Leu Pro Leu Leu Leu Gly Leu Asn Ala Gly Ala Val Ile Asp Trp Pro
 20 25 30
 Thr Glu Glu Gly Lys Glu Val Trp Asp Tyr Val Thr Val Arg Lys Asp
 35 40 45
 Ala Tyr Met Phe Trp Trp Leu Tyr Tyr Ala Thr Asn Ser Cys Lys Asn
 50 55 60

101

Phe Ser Glu Leu Pro Leu Val Met Trp Leu Gln Gly Gly Pro Gly Gly
 65 70 75 80
 Ser Ser Thr Gly Phe Gly Asn Phe Glu Glu Ile Gly Pro Leu Asp Ser
 85 90 95
 Asp Leu Lys Leu Arg Lys Thr Thr Trp Leu Gln Ala Ala Ser Leu Leu
 100 105 110
 Phe Val Asp Asn Pro Val Gly Thr Gly Phe Ser Tyr Val Asn Gly Ser
 115 120 125
 Gly Ala Tyr Ala Lys Asp Leu Ala Met Val Ala Ser Asp Met Met Val
 130 135 140
 Leu Leu Lys Thr Phe Phe Ser Cys His Lys Glu Phe Gln Thr Val Pro
 145 150 155 160
 Phe Tyr Ile Phe Ser Glu Ser Tyr Gly Gly Lys Met Ala Ala Gly Ile
 165 170 175
 Gly Leu Glu Leu Tyr Lys Ala Ile Gln Arg Gly Thr Ile Lys Cys Asn
 180 185 190
 Phe Ala Gly Val Ala Leu Gly Asp Ser Trp Ile Ser Pro Val Asp Ser
 195 200 205
 Val Leu Ser Trp Gly Pro Tyr Leu Tyr Ser Met Ser Leu Leu Glu Asp
 210 215 220
 Lys Gly Leu Ala Glu Val Ser Lys Val Ala Glu Gln Val Leu Asn Ala
 225 230 235 240
 Val Asn Lys Gly Leu Tyr Arg Glu Ala Thr Glu Leu Trp Gly Lys Ala
 245 250 255
 Glu Met Ile Ile Glu Gln Asn Thr Asp Gly Val Asn Phe Tyr Asn Ile
 260 265 270
 Leu Thr Lys Ser Thr Pro Thr Ser Thr Met Glu Ser Ser Leu Glu Phe
 275 280 285
 Thr Gln Ser His Leu Val Cys Leu Cys Gln Arg His Val Arg His Leu
 290 295 300
 Gln Arg Asp Ala Leu Ser Gln Leu Met Asn Gly Pro Ile Arg Lys Lys
 305 310 315 320
 Leu Lys Ile Ile Pro Glu Asp Gln Ser Trp Gly Gly Gln Ala Thr Asn
 325 330 335
 Val Phe Val Asn Met Glu Glu Asp Phe Met Lys Pro Val Ile Ser Ile
 340 345 350
 Val Asp Glu Leu Leu Glu Ala Gly Ile Asn Val Thr Val Tyr Asn Gly
 355 360 365
 Gln Leu Asp Leu Ile Val Asp Thr Met Gly Gln Glu Ala Trp Val Arg

102

370 375 380
 Lys Leu Lys Trp Pro Glu Leu Pro Lys Phe Ser Gln Leu Lys Trp Lys
 385 390 395 400
 Ala Leu Tyr Ser Asp Pro Lys Ser Leu Glu Thr Ser Ala Phe Val Lys
 405 410 415
 Ser Tyr Lys Asn Leu Ala Phe Tyr Trp Ile Leu Lys Ala Gly His Met
 420 425 430
 Val Pro Ser Asp Gln Gly Asp Met Ala Leu Lys Met Met Arg Leu Val
 435 440 445
 Thr Gln Gln Glu
 450

<210> 128
 <211> 84
 <212> PRT
 <213> Homo sapiens

<400> 128
 Val Arg Gly Gly Gly His Ser Arg Pro Leu Ser Ala Leu Glu Glu Arg
 1 5 10 15
 Lys Thr Asp Ser Tyr Arg Tyr Pro Arg Thr Gly Ser Lys Asn Pro Lys
 20 25 30
 Ile Ala Leu Lys Leu Ala Glu Phe Gln Thr Asp Ser Gln Gly Lys Ile
 35 40 45
 Val Ser Thr Gln Glu Lys Glu Leu Val Gln Pro Phe Ser Ser Leu Phe
 50 55 60
 Pro Lys Val Glu Tyr Ile Ala Arg Ala Gly Trp Thr Arg Asp Gly Lys
 65 70 75 80
 Tyr Ala Trp Ala

<210> 129
 <211> 349
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (183)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (191)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (192)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (334)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (344)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (345)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (348)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 129
 Met Phe Leu Asp Arg Pro Gln Gln Trp Leu Gln Leu Val Leu Leu Pro
 1 5 10 15
 Pro Ala Leu Phe Ile Pro Ser Thr Glu Asn Glu Glu Gln Arg Leu Ala
 20 25 30
 Ser Ala Arg Ala Val Pro Arg Asn Val Gln Pro Tyr Val Val Tyr Glu
 35 40 45
 Glu Val Thr Asn Val Trp Ile Asn Val His Asp Ile Phe Tyr Pro Phe
 50 55 60
 Pro Gln Ser Glu Gly Glu Asp Glu Leu Cys Phe Leu Arg Ala Asn Glu
 65 70 75 80
 Cys Lys Thr Gly Phe Cys His Leu Tyr Lys Val Thr Ala Val Leu Lys
 85 90 95
 Ser Gln Gly Tyr Asp Trp Ser Glu Pro Phe Ser Pro Gly Glu Asp Glu
 100 105 110
 Phe Lys Cys Pro Ile Lys Glu Glu Ile Ala Leu Thr Ser Gly Glu Trp
 115 120 125
 Glu Val Leu Ala Arg His Gly Ser Lys Ile Trp Val Asn Glu Glu Thr
 130 135 140
 Lys Leu Val Tyr Phe Gln Gly Thr Lys Asp Thr Pro Leu Glu His His
 145 150 155 160
 Leu Tyr Val Val Ser Tyr Glu Ala Ala Gly Glu Ile Val Arg Leu Thr
 165 170 175

Thr Pro Gly Phe Ser His Xaa Cys Ser Met Ser Gln Asn Phe Xaa Xaa
 180 185 190
 Phe Val Ser His Ile Thr Ala Gln Val Ala Ala Ala Ser Ala Gly Asn
 195 200 205
 Gln Ala Gly Gly Thr Glu Trp Pro Ala Gly Pro Ser Glu Ala Leu Cys
 210 215 220
 Pro Ala Gln Arg Trp Pro Ala Pro Arg Ser Arg Cys Leu His Arg Pro
 225 230 235 240
 Asp Ala Phe Tyr Pro Phe Leu Asn Ala Leu Gly Phe Tyr Val Arg Cys
 245 250 255
 Phe Leu Val Ala Glu Thr Glu Arg Trp Trp Ser Arg Ala Ser Pro Ser
 260 265 270
 Ser Pro Arg Leu Leu Gly Gly Gly Gly His Thr Leu Met Gly Thr Gly
 275 280 285
 Glu Ala Arg Arg Asp Ser Glu Glu Arg Ala Ala Phe Arg Leu Gly Leu
 290 295 300
 Pro Val Thr Ser Gln Ser Pro Gly Pro Ala Ser His Arg Pro Gln His
 305 310 315 320
 Pro Ser Met Gln Leu Pro Val Pro Pro Gly Gln Pro Pro Xaa Leu Asp
 325 330 335
 Val Cys Val Leu Phe Gly Gly Xaa Xaa Phe Ile Xaa Ile
 340 345

<210> 130
 <211> 177
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (121)
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>
 <221> SITE
 <222> (122)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 130
 Met Lys Val Leu Ala Thr Ser Phe Val Leu Gly Ser Leu Gly Leu Ala
 1 5 10 15
 Phe Tyr Leu Pro Leu Val Val Thr Thr Pro Lys Thr Leu Ala Ile Pro
 20 25 30
 Glu Lys Leu Gln Glu Ala Val Gly Lys Val Ile Ile Asn Ala Thr Thr

105

35		40		45
Cys Thr Val Thr Cys Gly Leu Gly Tyr Lys Glu Glu Thr Val Cys Glu				
50		55		60
Val Gly Pro Asp Gly Val Arg Arg Lys Cys Gln Thr Arg Arg Leu Glu				
65		70		80
Cys Leu Thr Asn Trp Ile Cys Gly Met Leu His Phe Thr Ile Leu Ile				
	85		90	95
Gly Lys Glu Phe Glu Leu Ser Cys Leu Ser Ser Asp Ile Leu Glu Phe				
	100		105	110
Gly Gln Glu Ala Phe Arg Phe Thr Xaa Xaa Leu Ala Arg Gly Val Ile				
	115		120	125
Ser Thr Asp Asp Glu Val Phe Lys Pro Phe Gln Ala Asn Ser His Phe				
	130		135	140
Val Lys Phe Lys Tyr Ala Gln Glu Tyr Asp Ser Gly Thr Tyr Arg Cys				
145		150		160
Asp Val Gln Leu Val Lys Asn Leu Arg Leu Val Lys Ser Ser Ile Leu				
	165		170	175

Gly

<210> 131
 <211> 131
 <212> PRT
 <213> Homo sapiens

<400> 131
 Asn Ile Phe Leu Glu Trp Ile Leu Arg Arg Ile Leu Ser Leu Trp Arg
 1 5 10 15
 Gly Thr Phe Leu Met His Gly Arg Ala Gly Val Asn Arg Ile Ser Tyr
 20 25 30
 Trp Pro Ala Asp Pro Glu Ile Ser Leu Leu Thr Glu Ala Ser Ser Ser
 35 40 45
 Glu Asp Ala Lys Leu Asp Ala Lys Ala Val Glu Arg Leu Lys Ser Asn
 50 55 60
 Ser Arg Ala His Val Cys Val Leu Leu Gln Pro Leu Val Cys Tyr Met
 65 70 75 80
 Val Gln Phe Val Glu Glu Thr Ser Tyr Lys Cys Asp Phe Ile Gln Lys
 85 90 95
 Ile Thr Lys Thr Leu Pro Asp Ala Asn Thr Asp Phe Tyr Tyr Glu Cys
 100 105 110
 Lys Gln Glu Arg Ile Lys Glu Tyr Glu Met Leu Lys Lys Lys Lys

106

115

120

125

Lys Lys Thr
130

<210> 132

<211> 180

<212> PRT

<213> Homo sapiens

<400> 132

Met Thr Ser Val Ser Gln Ala Ser Leu Asp Val Ser Met Ile Ile Ile
1 5 10 15

Ile Ser Leu Gly Ala Ile Cys Ala Val Leu Leu Val Ile Met Val Leu
20 25 30

Phe Ala Thr Arg Cys Asn Arg Glu Lys Lys Asp Thr Arg Ser Tyr Asn
35 40 45

Cys Arg Val Ala Glu Ser Thr Tyr Gln His His Pro Lys Arg Pro Ser
50 55 60

Arg Gln Ile His Lys Gly Asp Ile Thr Leu Val Pro Thr Ile Asn Gly
65 70 75 80

Thr Leu Pro Ile Arg Ser His His Arg Ser Ser Pro Ser Ser Ser Pro
85 90 95

Thr Leu Glu Arg Gly Gln Met Gly Ser Arg Gln Ser His Asn Ser His
100 105 110

Gln Ser Leu Asn Ser Leu Val Thr Ile Ser Ser Asn His Val Pro Glu
115 120 125

Asn Phe Ser Leu Glu Leu Thr His Ala Thr Pro Ala Val Glu Arg Leu
130 135 140

Ser Ala Ser Phe Asn Ala Ser Pro Gly Ala Ile Ser Ala Lys Thr Lys
145 150 155 160

Phe Ser Arg Lys Gln Ile Phe Gln Glu Leu Gln Ile Cys Pro Ser Arg
165 170 175

His Gly Gln Ile
180

<210> 133

<211> 200

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (140)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (155)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (165)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (173)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (194)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (196)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 133

Met	Ala	Val	Tyr	Val	Gly	Met	Leu	Arg	Leu	Gly	Arg	Leu	Cys	Ala	Gly
1				5					10					15	

Ser	Ser	Gly	Val	Leu	Gly	Ala	Arg	Ala	Ala	Leu	Ser	Arg	Ser	Trp	Gln
		20				25						30			

Glu	Ala	Arg	Leu	Gln	Gly	Val	Arg	Phe	Leu	Ser	Ser	Arg	Glu	Val	Asp
	35					40						45			

Arg	Met	Val	Ser	Thr	Pro	Ile	Gly	Gly	Leu	Ser	Tyr	Val	Gln	Gly	Cys
	50					55					60				

Thr	Lys	Lys	His	Leu	Asn	Ser	Lys	Thr	Val	Gly	Gln	Cys	Leu	Glu	Thr
65					70					75					80

Thr	Ala	Gln	Arg	Val	Pro	Glu	Arg	Glu	Ala	Leu	Val	Val	Leu	His	Glu
				85					90					95	

Asp	Val	Arg	Leu	Thr	Phe	Ala	Gln	Leu	Lys	Glu	Glu	Val	Asp	Lys	Ala
		100						105					110		

Ala	Ser	Gly	Leu	Leu	Ser	Ile	Gly	Leu	Cys	Lys	Gly	Asp	Arg	Leu	Gly
		115					120					125			

Met	Trp	Gly	Pro	Asn	Ser	Tyr	Ala	Trp	Val	Leu	Xaa	Gln	Leu	Ala	Thr
	130					135					140				

Gly	Gln	Ala	Gly	Ile	Ile	Leu	Val	Ser	Val	Xaa	Pro	Ala	Tyr	Gln	Ala
145					150					155					160

Met	Glu	Trp	Ser	Xaa	Ser	Ser	Lys	Lys	Trp	Ala	Ser	Xaa	Ala	Leu	Val
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

108

165

170

175

Val Pro Lys Gln Phe Lys Thr Lys His Asn Thr Thr Phe Leu Lys Gln
 180 185 190

Ile Xaa Pro Xaa Trp Arg Met Pro
 195 200

<210> 134

<211> 141

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (140)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 134

Met Gly Pro Arg Gly Cys Ala Leu Ala His Ser Leu Leu Pro Leu Leu
 1 5 10 15

Cys Gln His Val Trp Thr Ser Pro Arg Tyr Cys Arg Gln Cys Thr Arg
 20 25 30

Glu Pro Arg His Cys Cys Pro Ala Pro Ala Ser Ala Gly Val Gln Tyr
 35 40 45

Met Cys Ala Tyr Gly Cys His His Pro Thr Phe Ala Gly Val Tyr Thr
 50 55 60

Pro Ser His Thr Thr Val Ala Thr Ser Ile Cys Thr Gln Thr Pro Pro
 65 70 75 80

His Gln Cys Cys Trp Ser Glu His Thr His Val Val Ser Thr Thr Pro
 85 90 95

Leu Leu Pro Ala Tyr Met His Met Ser Met Asp Pro Ala Ala Thr Thr
 100 105 110

Gln Met Lys Cys Phe Cys Arg His Pro Ile Arg Ala Phe Leu Pro Val
 115 120 125

Glu Trp Glu His Leu Ser Pro Phe Asn Thr Ala Xaa Ala
 130 135 140

<210> 135

<211> 201

<212> PRT

<213> Homo sapiens

<400> 135

His Met Asp Phe Ile Val Ala Ala Ser Asn Leu Arg Ala Glu Asn Tyr
 1 5 10 15

Asp Ile Pro Ser Ala Asp Arg His Lys Ser Lys Leu Ile Ala Gly Lys

109

20					25					30									
Ile	Ile	Pro	Ala	Ile	Ala	Thr	Thr	Thr	Ala	Ala	Val	Val	Gly	Leu	Val				
35					40					45									
Cys	Leu	Glu	Leu	Tyr	Lys	Val	Val	Gln	Gly	His	Arg	Gln	Leu	Asp	Ser				
50					55					60									
Tyr	Lys	Asn	Gly	Phe	Leu	Asn	Leu	Ala	Leu	Pro	Phe	Phe	Gly	Phe	Ser				
65					70					75					80				
Glu	Pro	Leu	Ala	Ala	Pro	Arg	His	Gln	Tyr	Tyr	Asn	Gln	Glu	Trp	Thr				
85					90					95									
Leu	Trp	Asp	Arg	Phe	Glu	Val	Gln	Gly	Leu	Gln	Pro	Asn	Gly	Glu	Glu				
100					105					110									
Met	Thr	Leu	Lys	Gln	Phe	Leu	Asp	Tyr	Phe	Lys	Thr	Glu	His	Lys	Leu				
115					120					125									
Glu	Ile	Thr	Met	Leu	Ser	Gln	Gly	Val	Ser	Met	Leu	Tyr	Ser	Phe	Phe				
130					135					140									
Met	Pro	Ala	Ala	Lys	Leu	Lys	Glu	Arg	Leu	Asp	Gln	Pro	Met	Thr	Glu				
145					150					155					160				
Ile	Val	Ser	Arg	Val	Ser	Lys	Arg	Lys	Leu	Gly	Arg	His	Val	Arg	Ala				
165					170					175									
Leu	Val	Leu	Glu	Leu	Cys	Cys	Asn	Asp	Glu	Ser	Gly	Glu	Asp	Val	Glu				
180					185					190									
Val	Pro	Tyr	Val	Arg	Tyr	Thr	Ile	Arg											
195					200														

<210> 136

<211> 52

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (46)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 136

Met	Val	Pro	Ile	Phe	Leu	Leu	Lys	Cys	Leu	Leu	Leu	His	Val	Pro	Leu
1				5					10					15	

Cys	Met	Ser	Ser	Asn	Leu	Ser	Phe	His	Ser	Ser	His	His	Leu	His	Ile
		20					25						30		

Phe	Leu	Pro	Ser	Phe	Ser	Ser	His	Leu	Pro	Arg	Pro	Leu	Xaa	Ile	Pro
		35					40					45			

Pro	Leu	Ser	Pro
		50	

110

<210> 137
 <211> 215
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (116)
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 137

Met	Glu	Leu	Ser	Cys	Pro	Gly	Ser	Arg	Cys	Pro	Val	Gln	Glu	Gln	Arg	
1				5					10					15		
Ala	Arg	Trp	Glu	Arg	Lys	Arg	Ala	Cys	Thr	Ala	Arg	Glu	Leu	Leu	Glu	
			20					25					30			
Thr	Glu	Arg	Arg	Tyr	Gln	Glu	Gln	Leu	Gly	Leu	Val	Ala	Thr	Tyr	Phe	
		35					40					45				
Leu	Gly	Ile	Leu	Lys	Ala	Lys	Gly	Thr	Leu	Arg	Pro	Pro	Glu	Arg	Gln	
	50					55					60					
Ala	Leu	Phe	Gly	Ser	Trp	Glu	Leu	Ile	Tyr	Gly	Ala	Ser	Gln	Glu	Leu	
65					70					75					80	
Leu	Pro	Tyr	Leu	Glu	Gly	Gly	Cys	Trp	Gly	Gln	Gly	Leu	Glu	Gly	Phe	
			85						90					95		
Cys	Arg	His	Leu	Glu	Leu	Tyr	Asn	Gln	Phe	Ala	Ala	Asn	Ser	Glu	Arg	
			100					105					110			
Ser	Gln	Thr	Xaa	Leu	Gln	Glu	Gln	Leu	Lys	Lys	Asn	Lys	Gly	Phe	Arg	
		115					120					125				
Lys	Phe	Val	Arg	Leu	Gln	Glu	Gly	Arg	Pro	Glu	Phe	Gly	Gly	Leu	Gln	
	130					135					140					
Leu	Gln	Asp	Leu	Leu	Pro	Leu	Pro	Leu	Gln	Arg	Leu	Gln	Gln	Tyr	Glu	
145					150					155					160	
Asn	Leu	Val	Val	Ala	Leu	Ala	Glu	Asn	Thr	Gly	Pro	Asn	Ser	Pro	Asp	
			165						170					175		
His	Gln	Gln	Leu	Thr	Arg	Arg	Phe	Leu	Leu	Leu	Gly	Asn	Ala	Gly	Trp	
			180					185					190			
Arg	Leu	Pro	Leu	Leu	Tyr	Ser	Phe	Leu	Ile	Leu	Thr	Ser	Asn	Asn	Val	
		195					200					205				
Trp	Tyr	Asp	Pro	Ile	Phe	His										
	210					215										

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<212> PRT

<213> Homo sapiens

<400> 138

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Met Ala Leu Leu Ala Asp Cys Ala Leu Pro Pro Glu Leu Arg Gly Asp
 1           5           10           15

Leu Trp Glu Leu Pro Phe Pro Cys Pro Asp Gly Phe Asn Ser Cys Pro
          20           25           30

Asp Ile Cys Phe Arg Val Ala Gly Cys Ser Phe Leu Cys His Lys Ala
          35           40           45

Phe Phe Cys Gly Arg Ser Asp Tyr Phe Arg Ala Leu Leu Asp Asp His
 50           55           60

Phe Arg Glu Ser Glu Glu Pro Ala Thr Ser Gly Gly Pro Pro Ala Val
 65           70           75           80

Thr Leu His Gly Ile Ser Pro Asp Val Phe Thr His Val Leu Tyr Tyr
          85           90           95

Met Tyr Ser Asp His Thr Glu Leu Ser Pro Glu Ala Ala Tyr Asp Val
          100          105          110

Leu Ser Val Ala Asp Met Tyr Leu Leu Pro Gly Leu Lys Arg Leu Cys
          115          120          125

Gly Arg Ser Leu Ala Gln Met Leu Asp Glu Asp Thr Val Val Gly Val
          130          135          140

Trp Arg Val Ala Lys Leu Phe Arg Leu Ala Arg Leu Glu Asp Gln Cys
          145          150          155          160

Thr Glu Tyr Met Ala Lys Val Ile Glu Lys Leu Val Glu Arg Glu Asp
          165          170          175

Phe Val Glu Ala Val Lys Glu Glu Ala Ala Ala Val Ala Ala Arg Gln
          180          185          190

Glu Thr Asp Ser Ile Pro Leu Val Asp Asp Ile Arg Phe His Val Ala
          195          200          205

Ser Thr Val Gln Thr Tyr Ser Ala Ile Glu Glu Ala Gln Gln Arg Leu
          210          215          220

Arg Ala Leu Glu Asp Leu Leu Val Ser Ile Gly Leu Asp Cys
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<210> 139

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<212> PRT

<213> Homo sapiens

<220>

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<222> (180)

<223> Xaa equals any of the naturally occurring L-amino acids

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Phe	Ser	Val	Val	Thr	Trp	Met	Ile	Phe	Ile	Ile	Ser	Gln	Asn	Phe	Thr
		20					25						30		

Lys	Leu	Trp	Ser	Ala	Leu	Asn	Leu	Ser	Ile	Ser	Val	His	Tyr	Trp	Asn
		35				40						45			

Asn	Ser	Ala	Lys	Ser	Leu	Phe	Pro	Lys	Thr	Ser	Leu	Ile	Pro	Leu	Lys
	50					55					60				

Pro	Leu	Thr	Glu	Thr	Glu	Leu	Arg	Ile	Lys	Glu	Ile	Ile	Glu	Lys	Leu
65					70					75					80

Asp	Gln	Gln	Ile	Pro	Pro	Arg	Pro	Phe	Thr	His	Val	Asn	Thr	Thr	Thr
				85					90					95	

Ser	Ala	Thr	His	Ser	Thr	Ala	Thr	Ile	Leu	Asn	Pro	Arg	Asp	Thr	Tyr
		100						105					110		

Cys	Arg	Gly	Asp	Gln	Leu	Asp	Ile	Leu	Leu	Glu	Val	Arg	Asp	His	Leu
		115					120					125			

Gly	Gln	Arg	Lys	Gln	Tyr	Gly	Gly	Asp	Phe	Leu	Arg	Ala	Arg	Met	Ser
	130					135					140				

Phe	Pro	Ala	Leu	Thr	Ala	Gly	Ala	Ser	Gly	Lys	Val	Met	Asp	Phe	Thr
145					150					155					160

Met	Ala	Pro	Thr	Trp	Gln	Leu	His	Ser	Gly	Leu	Gly	Gly	Pro	Gly	Leu
				165					170					175	

Pro	Gly	Ser	Xaa	Xaa	Tyr	Ser	Pro	Gln	Val	Glu	Gly	Ala	Xaa	Gly	
			180					185					190		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/00544

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.5, 24.31; 530/350, 300; 435/6, 69.1, 252.3, 320.1, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database: EST; Accession NO: AI245696; NCI-CGAP; "Homo sapiens cDNA clone NCI_CGAP_Kid3, 3' similar to contain Alu repetitive element"; 04 November 1998; having 100% sequence identity to SEQ ID NO: 11 over 451 nucleotides; vector: pT7T3D-pac; host cell: DH10B; see entire document.	1-10, 21
X	Database: A_Geneseq_0401; Accession NO: Y41726; WOOD et al.; "Human PRO944 protein"; 07 December 1999; having 50.4% sequence identity to SEQ ID NO: 68; see entire document.	1-7, 21

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

17 JUNE 2001

Date of mailing of the international search report

05 SEP 2001

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Commissioner of Patents and Trademarks
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Authorized officer

RITA MITRA

Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database: A_Geneseq_0401; Accession NO: Y04143; GOODEARL et al.; "Human Tango-73 protein"; 15 June 1999; having 50.4% sequence identity to SEQ ID NO: 68; see entire document.	1-10, 21
X	Database: A_Geneseq_0401; Accession NO: W99653; HOSIER et al.; "Human senescence factor p23 protein"; 21 May 1999; having 50.4% sequence identity to SEQ ID NO: 68; see entire document.	1-7, 21
X	Database: A_Geneseq_0401; Accession NO: Y68679; HILLMAN et al.; "A human molecule associated with apoptosis 2 (MAPOP-2)"; 05 May 2000; having 50.4% sequence identity to SEQ ID NO: 68; see entire document.	1-7, 21
X	Database: SwissProt_39; Accession NO: O95832; NCBI_TaxID=9606; "Claudin-1"; 30 May 2000; having 50.4% sequence identity to SEQ ID NO: 68; see entire document.	1-7, 21
X	MORITA et al. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc. Natl. Acad. Sci. USA. January 1999, Vol. 96 (2), pages 511-516, Fig 1 for claudin family sequence alignment, see page 512, col 1 for vector: pCAGGS and for host cell: MDCK cells under "Materials and Methods", see page 513, col. 2 for transfection into cultured MDCK cells; see entire document.	1-10, 14, 15, 21

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10, 14, 15 and 21, all in part

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C07H 21/04, 21/02; C07K 5/00, 14/00; C12Q 1/68; C12N 1/21, 15/63, 15/85, 15/86

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

538/23.1, 23.5, 24.31; 530/350, 300; 435/6, 69.1, 252.3, 320.1, 325

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

Sequence Search (Database: GenEmbl, N_Geneseq_0401, Issued_Patent_NA, EST, A_Geneseq_0401, Issued_Patents_AA, PIR_67, SwissProt_39, SPTREMBL_15)

EAST (Database: USPT, EPO, JPO, Derwent)

STN (Database: biosis caplus, embase, medline, scisearch)

search Terms: secreted protein, claudin, four-transmembrane domain protein, polynucleotides, human kidney

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Groups 1-57, claims 1-10, 14, 15 and 21, all in part, drawn to an isolated nucleic acid of SEQ ID NO X or a peptide of SEQ ID NO: Y, wherein X and Y are values that correlate to those listed in Table 1, and correspond to one of the cDNA Clone IDs, respectively. For examples,

If group 1 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein X is 11 and Y is 68.

If group 2 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein X is 12 and Y is 69.

Groups 58-114, claims 11, 12 and 16, all in part, each group directed to a peptide of SEQ ID NO: Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 58 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein Y is 68.

If group 59 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein Y is 69.

Groups 115-171, claim 13, in part, drawn to an isolated antibody which binds to a protein with SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 115 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein Y is 68.

If group 116 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein Y is 69.

Groups 172-228, claim 17, in part, drawn to a method for preventing, treating or ameliorating an undefined medical condition by administering a polypeptide of SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 172 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein Y is 68.

If group 173 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein Y is 69.

Groups 229-285, claim 17, in part, drawn to a method for preventing, treating or ameliorating an undefined medical condition by administering a polynucleotide of SEQ ID NO X encoding a protein of SEQ ID NO Y, wherein X and Y correlate to one of those listed in Table 1, and correspond to one of the cDNA Clone IDs, respectively. For examples,

If group 229 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein X is 11

and Y is 68.

If group 280 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein X is 12 and Y is 69.

Groups 286-342, claim 18, in part, drawn to a method of diagnosis of an undefined pathological condition by determining the presence or absence of a mutation in a polynucleotide of SEQ ID NO X, wherein X correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 286 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein X is 11.

If group 287 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein X is 12.

Groups 343-399, claim 19, in part, drawn to a method of diagnosis of an undefined pathological condition by determining the presence or amount of expression of the polypeptide of SEQ ID NO y, wherein y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 343 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein y is 68.

If group 344 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein y is 69.

Groups 400-456, claim 20, in part, drawn to a method of identifying a binding partner to a polypeptide defined by SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 400 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein Y is 68.

If group 401 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein Y is 69.

Groups 457-513, claim 22, in part, drawn to a method of identifying an activity in a biological assay by identification of the protein in the supernatant wherein the cell expresses a polypeptide encoded by SEQ ID NO X, wherein X correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 457 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein X is 11.

If group 458 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein X is 12.

Groups 514-570, claim 23, in part, each group directed to a peptide produced by the method for the identifying a binding partner to a polypeptide defined by SEQ ID NO: Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 514 is elected, this correlates to Gene No 1, cDNA clone ID HFKLX38 of Table 1, wherein Y is 68.

If group 515 is elected, this correlates to Gene No 2, cDNA clone ID HUVFH14, wherein Y is 69.

The inventions listed as Groups 1-570 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The polynucleotides and polypeptides of each of the clones in Table 1 are unrelated, each to the other. The polynucleotide sequences encode structurally distinct polypeptides and do not share a special technical feature. Furthermore, the technical feature that links the DNA, protein, antibody, methods of CDNA clone HFKLX38(see Table 1)is not a contribution over the prior art. See the various documents cited in the search report. Thus the technical feature of the polynucleotide sequence is not special and the groups are not so linked under PCT Rule 13.1. Additionally the claimed methods produce different products and/or different results which are not coextensive and which do not share the same technical feature.